



CAMBRIDGE UNIVERSITY PRESS

LONDON: FETTER LANE, E.C. 4

also

H. K. LEWIS & CO., LTD., 136, GOWER STREET, LONDON, W.C. 1

CHICAGO: THE UNIVERSITY OF CHICAGO PRESS

(AGENTS FOR THE UNITED STATES)

BOMBAY, CALCUTTA, MADRAS: MACMILLAN & CO., LTD.

TOKYO: THE MARUZEN-KABUSHIKI-KAISHA

BIOLOGICAL  
REVIEWS  
AND  
BIOLOGICAL PROCEEDINGS  
OF THE  
*Cambridge Philosophical Society*

✧—————✧  
Edited  
by  
H. MUNRO FOX

✧—————✧

VOLUME IV  
1929

CAMBRIDGE  
AT THE UNIVERSITY PRESS  
1929



PRINTED IN GREAT BRITAIN

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Erratum to the article by A. M. DALCQ in vol. III, page 181, line 18. *For* On sait que . . .  
*read* On sait que dans les spermatocytes de la sauterelle CHAMBERS (1925) a vu se dessiner  
les chromosomes grâce à la simple pénétration de l'aiguille à microdissection dans le  
noyau, mais cela uniquement dans les spermatocytes qui étaient sur le point de se diviser.



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DER ISOELEKTRISCHE PUNKT VON ZELLEN  
UND GEWEBEN

VON DR. HANS PFEIFFER (BREMEN).

(Received July 30, 1928.)

(With Seven Text-figures.)

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## A. ZUR EINFÜHRUNG.

§ 1. *Begründung des folgenden Berichts.* Schon häufig sind in den letzten Jahren pflanzliche und tierische Gewebe in ihrem Verhalten mit Ampholyten verglichen worden, und verschiedentlich hat dieser Vergleich auch in dem erstrebten Nachweise eines IEP<sup>1</sup> oder eines dafür gehaltenen Wendepunktes gegipfelt. Dabei hat sich allerdings ein *Wechsel in dem früheren Begriffsumfange* des IEP eingestellt, auf welchen die betr. Autoren nur selten aufmerksam geworden sind. Beides soll in den folgenden Ausführungen so knapp wie angängig betrachtet werden. Es wird also zuerst untersucht werden, in welcher Einschränkung man eigentlich nur von einem IEP reden dürfte. Michaelis, der auf diesem Gebiete durch eigene und durch Arbeiten seiner Schüler bahnbrechend gewirkt hat, verwendet den Begriff zwar selber meist in dem abgewandelten Sinne. Wo. Pauli hat auch häufiger auf die alte Begriffsbegrenzung des IEP und auf die Anwendung der Bezeichnung auf das Neutralteilchen-( $\rho$ -) Maximum aufmerksam gemacht. Dass diese Gedanken aber noch nicht allgemein in die Literatur eingedrungen sind, berechtigt sicher zur Abfassung des folgenden Überblicks. Dieser wird sich weiter mit der verschiedenen beurteilten Frage der Existenz eines IEP oder eines ihm entsprechenden Wendepunktes beschäftigen, dabei allerdings sehr häufig auf die Ergänzungsbedürftigkeit unserer Kenntnisse hinzuweisen haben.

Bei dem erheblichen Umfange, den die zu erwähnende Literatur bereits erreicht hat, ist indessen eine gewisse *Auswahl der Zitate* ein unabweisliches Bedürfnis. Wir können uns dazu um so eher verstehen, als manche der besprochenen Fragen zusammen mit vielen andern Problemen in Form einer kurzen Literaturübersicht als "Wissenschaftlicher Forschungsbericht" <sup>(222)</sup> ebenfalls behandelt werden. Ohne diesen Bericht ständig zu zitieren, sei doch allgemein zur Ergänzung der angegebenen Literatur auf ihn verwiesen, ob auch der hier behandelte Abschnitt dort nur einen kleineren Anteil ausmacht. Um Raum zu sparen, ist auch von einer tabellarischen Übersicht über die heute zumeist diskutierten Werte für den IEP bestimmter Zellen abgesehen (siehe darüber <sup>(222)</sup>, pp. 57-62!), zumal die bisherigen Zahlen nach unserer Auffassung nicht ohne weiteres vergleichbar sein werden.

B. DER BEGRIFF DES IEP IN BEZIEHUNG ZU DEM DES  $\rho$ -MAXIMUMS.(a) *Elementare Ableitung des Begriffsumfanges beider.*

§ 2. *Die Eiweisse als Ampholyte und ihr IEP.* Der Begriff des IEP geht auf Hardy <sup>(80)</sup> zurück. Er konstatierte für einen deutlich begrenzten Säuregehalt einer

<sup>1</sup> IEP: isoelektrischer Punkt.

<sup>2</sup> Die petit gesetzten Ziffern verweisen auf die Nummern des angehängten Literaturverzeichnisses.

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Lösung von gekochtem Eiweiss den Verlust elektrophoretischer Wanderung und den Eintritt einer Ausflockung, nach weiterem Zusatz von nur wenig Säure oder Base aber zugleich mit erneuter Auflösung eine kathodisch bzw. anodisch gerichtete Wanderung der Teilchen. Bredig<sup>(25)</sup> hat die Eiweisskörper wegen ihres Aufbaues aus Aminosäuren als Ampholyte charakterisiert, die in saurer Lösung in ihrem Verhalten einer Base, in alkalischer aber einer Säure gleichen. Gegenüber der hauptsächlich von E. Fischer begründeten Auffassung der Polypeptidnatur der Eiweisse sind zwar neben der Säureamidbindung neuerdings noch andere Verkettungsweisen (Vorkommen zyklischer Pyrrolringe oder zyklischer Anhydride; Literatur s. H. Pfeiffer<sup>(222)</sup>, p. 61) angenommen worden, die aber keineswegs gegen den Ampholytcharakter verstossen (Wo. Pauli<sup>(205)</sup>, p. 190). Eine gewisse Komplizierung ist allerdings durch die Annahme von Zwitterionen mit zwei verschiedenen Ladungen am gleichen Radikalkomplex (G. Bredig, N. Bjerrum, L. Ebert usw.) neben den sonst schon angenommenen positiven und negativen Ionen und den Neutralteilchen gegeben. Die Existenz des Zwitterions ist durch die Elektrophorese wegen seiner an sich gegebenen Elektroneutralität nicht nachweisbar; seine Menge ist sicher von der hydrolytischen Spaltung des Salzes (§ 22) mit der schwachen Base der Aminosäure abhängig.

§ 3. *Die analytische Bestimmung des  $\rho$ -Maximums.* Die heute am meisten verbreitete Theorie des IEP, die wir hauptsächlich Michaelis und seinen Schülern (vgl. (169), (171), (172), (174), (175), (176) pp. 56, 58, 241; (177), (177a), (180) bis (182); ferner: (79) p. 71; (135) p. 246; (101) p. 96; (31) pp. 32, 306, 338 und die dort angeführte Literatur!) verdanken, charakterisiert diesen Wert als das Maximum der Kurve des Dissoziationsrestes  $\rho$ . Setzen wir  $A$  für die Gesamtquantität des Ampholyten,  $A'$  für das Kation,  $A''$  für das Anion,  $x$  für den undissoziierten Anteil,  $k_s$  für die Säuredissoziationskonstante,  $k_b$  für die Basendissoziationskonstante,  $k_w$  für die Dissoziationskonstante des Wassers, so gelten die Gleichungen:

$$C_{A'} = \frac{C_x \cdot k_s}{C_H} \text{ und } C_{A''} = \frac{C_x \cdot k_b}{C_{OH}} \quad \dots\dots\dots(1),$$

$$C_x = \frac{C_A - C_{A'} - C_{A''}}{C_A}.$$

$$\rho = \frac{x}{A} \quad \dots\dots\dots(2).$$

$$\rho = \frac{1}{1 + \frac{k_s}{C_H} + \frac{k_b}{k_w} \cdot C_H} \quad \dots\dots\dots(3).$$

Das Maximum von  $\rho$  lässt sich analytisch leichter durch das Minimum von  $1/\rho$  darstellen. Aus

$$\frac{1}{\rho} = 1 + \frac{k_s}{C_H} + \frac{k_b}{k_w} \cdot C_H \quad \dots\dots\dots(4)$$

folgt nach Differenzieren:

$$\frac{d(1/\rho)}{dC_H} = -\frac{k_s}{(C_H)^2} + \frac{k_b}{k_w} \quad \dots\dots\dots(5).$$



Setzen wir den Wert (5) gleich Null, so resultiert das Minimum von  $1/\rho$  oder das Maximum von  $\rho$ , und man erhält für die  $C_H$  im Maximum von  $\rho$ :

$$C_H \text{ bei } \rho_{\max.} = \sqrt{\frac{k_s}{k_b} \cdot k_w} \quad \dots\dots\dots(6),$$

und für die  $C_{OH}$  dementsprechend:

$$\frac{k_w}{C_H} = \frac{k_w}{\sqrt{\frac{k_s}{k_b} \cdot k_w}},$$

$$\text{d. h.:} \quad C_{OH} \text{ bei } \rho_{\max.} = \sqrt{\frac{k_b}{k_s} \cdot k_w} \quad \dots\dots\dots(7).$$

Die Ableitungen basieren auf der Annahme, dass die *ungeladene Form der Aminosäure in einer einzigen Modifikation* auftritt. Michaelis (177) p. 149 hat selber die Möglichkeit erwähnt, dass im ungeladenen Zustande zwei Tautomere  $\alpha'$  und  $\alpha''$  des Ampholyten vorliegen könnten, die positiven aber nur in einer ( $\alpha'$ ), die negativen in der andern ( $\alpha''$ ) auftreten. Dann würden die Gleichungen (1) übergehen in die Form:

$$C_{A'} = \frac{C_{\alpha'} \cdot k_s}{C_H} \text{ und } C_{A'} = \frac{C_{\alpha''} \cdot k_b}{C_{OH}} \quad \dots\dots\dots(8).$$

Durch Eckweiller, Noyes und Falk (48) ist unter Fortführung dieses Gedankens eine Abänderung der Formel (6) vorgeschlagen worden:

$$C_H \text{ bei } \rho_{\max.} = \sqrt{\frac{k_s}{k_b} \cdot k_w \cdot \frac{C_{A'}}{C_{\alpha'}} \cdot \frac{C_{\alpha''}}{C_{A'}}} \quad \dots\dots\dots(9).$$

Von Michaelis (176), p. 58) wird ihre Überlegung aber mit Recht zurückgewiesen, weil experimentell nie das Gleichgewicht zwischen den Ionen und je einer der Tautomeren des unelektrischen Moleküls bestimmt wird, sondern nur immer das Verhältnis zwischen den Ionen und den undissoziierten Molekülen überhaupt.

(b) *Folgerungen aus den Gleichungen für das  $\rho$ -Maximum.*

§ 4. *Die Gleichheit der Kat- und Anionen.* Aus den Gleichungen (6) und (7) ergeben sich mehrere Folgerungen. Zuerst erhält man aus den Gleichungen (1) für den IEP:

$$C_{A'} = C_{\alpha} \cdot \frac{k_s}{\sqrt{\frac{k_s}{k_b} \cdot k_w}} \text{ und } C_{A'} = C_{\alpha} \cdot \frac{k_b}{\sqrt{\frac{k_b}{k_s} \cdot k_w}} \quad \dots\dots\dots(10),$$

$$\text{oder:} \quad C_{A'} = C_{\alpha} \sqrt{\frac{k_s \cdot k_b}{k_w}} \text{ und } C_{A'} = C_{\alpha} \sqrt{\frac{k_s \cdot k_b}{k_w}} \quad \dots\dots\dots(11),$$

$$\text{folglich:} \quad C_{A'} = C_{A'} \quad \dots\dots\dots(12),$$

d. h. in Worten: *im  $\rho$ -Maximum ist die Konzentration der Anionen gleich jener der Kationen.*

Das bedeutet, dass  $H'$  und  $OH'$  in jenem Punkte in gleicher Menge abdissoziiert werden, *der Ampholyt die Azidität der Lösung also nicht beeinflusst. Im elektrophoretischen Gefälle erfolgt keine Wanderung.*

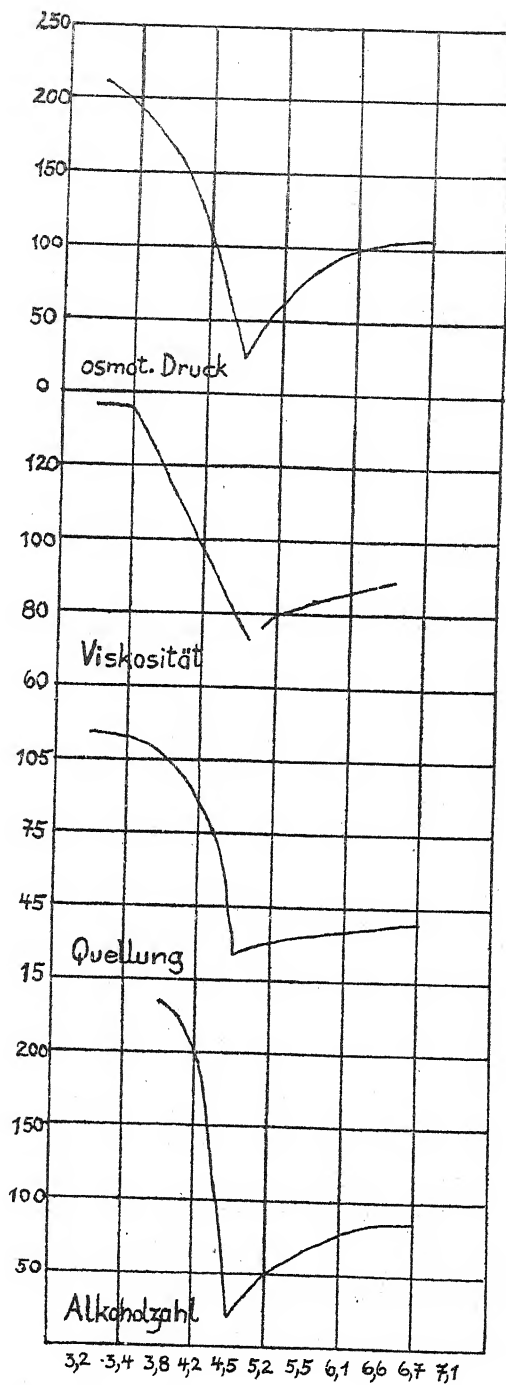


Fig. 1. Der Einfluss des Aziditätsgrades auf osmotischen Druck, Viskosität, Quellung und Alkoholzahl einer Eiweisslösung (Minimum dieser kolloidchemischen Eigenschaften in der Nähe des IEP und des Neutralteilchenmaximums).

§ 5. *Das Minimum der Kat- und Anionen.* Ferner lassen sich die Gleichungen (1) nach den Dissoziationskonstanten  $k_b$  und  $k_s$  auflösen:

$$k_b = \frac{C_{A'} \cdot C_{OH}}{C_w} \text{ und } k_s = \frac{C_{A'} \cdot C_{II}}{C_w} \quad \dots\dots\dots(13),$$

und aus den Gleichungen (11) folgt:

$$C_{A'} + C_{A''} = k_b \frac{C_w \cdot C_H}{k_w} + k_s \frac{C_w}{C_H} \quad \dots\dots\dots(14).$$

Der Ausdruck  $(C_{A'} + C_{A''})$  wird ein Minimum, sofern die Differentiation den Wert Null annimmt:

$$\frac{d(C_{A'} + C_{A''})}{dC_{II}} = 0, \quad \dots\dots\dots(15).$$

d. h. aber zugleich: wenn der Wert der  $C_{II}$  die Bedingungen der Gleichung (6) erfüllt. So folgt, dass die Summe der Konzentrationen der Kat- und Anionen im  $\rho$ -Maximum (IEP) ein Minimum bildet.

§ 6. *Weitere Minima.* Denken wir uns die Lösung eines Ampholyten in Berührung mit dem Bodenkörper, so ist sie bei jeder  $C_H$  an unelektrischen Molekülen gesättigt, wenn die Summe der Ionen ein Minimum hat. Dementsprechend ist dem Maximum der  $\rho$ -Kurve (IEP) auch eine minimale Löslichkeit eigentümlich, wie denn überhaupt alle auf den Beziehungen eines Ampholyten zum Lösungsmittel beruhenden Eigenschaften bei jenem Werte ein Minimum zeigen müssen (Fig. 1). Die letztere Folgerung ergibt sich auch aus der Vorstellung von Wo. Pauli, dass die Eiweissionen die Träger der Hydratation darstellen.

(c) *Ansätze zur kritischen Bestimmung des IEP und anderer Wendepunkte im Aziditätsverhalten von Ampholyten.*

§ 7. *Die Komplikationen bei Gegenwart undissoziierter Salz-moleküle.* Auf den Folgerungen der §§ 4-6 beruhen die gebräuchlichen Bestimmungen des IEP. Wie ersichtlich, sind die dabei angewandten Gleichungen nur für das Maximum des Dissoziationsrestes  $\rho$  abgeleitet worden. Zwar fällt bei totaler Dissoziation der Elektrolyte der Unterschied dieses Wertes gegenüber dem IEP fort. Doch treten bei Gegenwart undissoziierter Salz-moleküle Komplikationen der allgemeinen Dissoziationsprozesse auf. Die hier weiterhin gegebenen Darlegungen gelten zumeist nur für univalente Ionen. Wegen der weiteren Komplizierung bei Anwesenheit mehrwertiger Ionen muss auf die Originaluntersuchungen von Michaelis verwiesen werden.

Setzen wir bei Untersuchung der Verhältnisse in Säuren für das neben den  $H^+$  vorhandene Kation  $\kappa$  und für das aus ihm mit der Säure gebildete Salz  $\gamma$ , so resultiert (Michaelis (174)):

$$\text{bei ausbleibender Salzbildung: } \rho = \frac{1}{1 + \frac{k_s}{C_H}} \quad \dots\dots\dots(16),$$

und im Falle der Salzbildung:

$$\rho = \frac{1}{1 + k_s \left( \frac{C_\kappa}{C_\gamma} + 1 \right)} \quad \dots\dots\dots(17).$$

Die  $\rho$ -Kurve im letzteren Falle entspricht also einer solchen mit einer andern Säure mit der Dissoziationskonstanten  $k_s \left( \frac{C_\kappa}{C_\gamma} + 1 \right)$  ohne echte Salzbildung, d. h. bei graphischer Darstellung wird die  $\rho$ -Kurve um den Betrag  $-\log \left( \frac{C_\kappa}{C_\gamma} + 1 \right)$  horizontal verschoben (Fig. 2, 3).

Bei der Salzbildung schwacher Säuren mit bivalenten Ionen (s. o.!) tritt neben der Horizontalverschiebung ein steilerer Anstieg der Kurve ein. Die ähnlichen Ableitungen für Basen zu geben, mag hier zur Einschränkung des Raumes unterlassen bleiben.

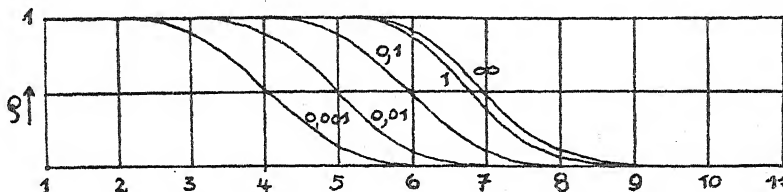


Fig. 2. Verlauf der Funktion  $\rho$  einer Säure mit der Dissoziationskonstanten  $k_s = 10^{-7}$  bei Anwesenheit eines Neutralsalzes mit einwertigem Kation in der Konzentration 0,1 und unter der Voraussetzung, dass die Dissoziationskonstante des aus der Säure und jenem Kation gebildeten Salzes den jeweils neben die Kurve gesetzten Wert zeigt. (Nach L. Michaelis.)

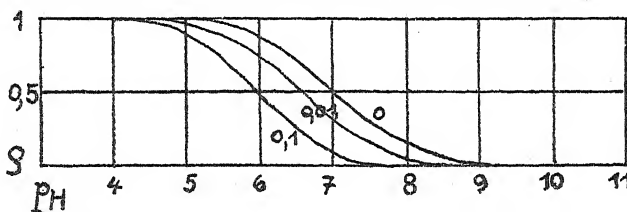


Fig. 3. Verlauf der Funktion  $\rho$  einer Säure der Dissoziationskonstanten  $k_s = 10^{-7}$  bei Anwesenheit eines Salzes mit einwertigem Kation in den an die Kurven gesetzten Konzentrationswerten unter der weiteren Voraussetzung, dass die Dissoziationskonstante des aus der Säure mit jenem Kation entstehenden Salzes dem Werte  $10^{-2}$  entspricht. (Nach L. Michaelis.)

Verständlicherweise tritt eine weitere Komplizierung bei *Ampholyten* ein. Von Michaelis ist dafür abgeleitet worden:

$$\rho = \frac{1}{1 + \frac{k_\delta}{k_w} \cdot C_H \left( 1 + \frac{C_\delta}{k_\delta} \right) + \frac{k_s}{C_H} \left( 1 + \frac{C_\gamma}{k_\gamma} \right)} \quad \dots\dots\dots(18),$$

wenn bezeichnen mögen:  $\delta$  das Ampholytsalz mit dem Metallkation,  $\gamma$  jenes mit dem Säureanion. Bei formaler Übereinstimmung der Kurve nach Gleichung (18) mit der  $\rho$ -Kurve für die Erscheinung ohne Salzbildung haben die Parameter doch eine andere Bedeutung. Aus dem Kurvenverlauf möchte etwa auf eine Erhöhung der beiden Dissoziationskonstanten des Ampholyten geschlossen werden. Weiter lässt sich mit Michaelis (vgl. auch die Gleichungen (6)!) für die  $C_H$  des  $\rho$ -Maximums finden:

$$C_H \text{ bei } \rho_{\max.} = \sqrt{\frac{k_s}{k_\delta} \cdot k_w \cdot \frac{\left( 1 + \frac{1}{k_\gamma} \right)}{\left( 1 + \frac{C_\delta}{k_\delta} \right)}} \quad \dots\dots\dots(19).$$

Die Veränderung der  $\rho$ -Kurve erstreckt sich danach in zweifacher Art; man findet neben der Horizontalverschiebung gleichzeitig eine Veränderung der Ordinatenhöhe (Fig. 4).

§ 8. Die Unabhängigkeit des IEP vom Stadium echter Salzbildung. Der Gedanke, dass die Maxima der Dissoziationsrestkurve mit dem IEP zusammenfallen müssten, kann wegen des geschilderten Verhaltens des  $\rho$ -Maximums (§ 7) nicht aufrecht erhalten werden, sobald die Definition des IEP als das Stadium gleich vieler positiver und negativer Ampholytionen und fehlender elektrophoretischer Bewegung beibehalten werden soll. Setzen wir für die Kat- und Anionen  $A'$  und  $A''$ , so können wir den Ladungsgrad  $\lambda$  (positiv oder negativ) definieren:

$$\lambda = C_{A'} - C_{A''} \quad \dots\dots\dots(20).$$

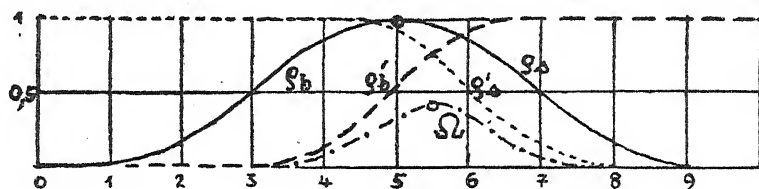


Fig. 4. Diagramm zur Veranschaulichung der Veränderung der  $\rho$ -Kurve bei Gegenwart von Salzen. (Nach L. Michaelis.) Die  $\rho$ -Kurve eines Ampholyten mit  $k_s = 10^{-7}$  und  $k_b = 10^{-11}$  wird abgeleitet aus der Kombination einer  $\rho_s$ -Kurve einer Säure mit der Dissoziationskonstanten  $k_s = 10^{-7}$  und einer  $\rho_b$ -Kurve einer Base mit  $k_b = \frac{k_w}{10^{-11}} = 10^{-3}$  (Lage des  $\rho_{\max}$ . bei pH 5,0). Wenn infolge Salzzusatzes der Ampholyt mit dem Anion ein Salz von der Konzentration 0,1 und mit dem Kation ein solches von der Konzentration 0,01 bildet, erfolgen Verschiebungen der Dissoziationskonstanten, nämlich von  $\rho_s$  nach  $\rho_s'$ , und von  $\rho_b$  nach  $\rho_b'$ . Dann resultiert die  $\rho$ -Kurve nach dem Salzzusatz aus der Kombination der Kurven für  $\rho_s'$  und  $\rho_b'$  (Verlauf der Kurve  $\Omega$ ).

Aus den Beziehungen des Massenwirkungsgesetzes (21)

$$C_{A'} = \frac{C_A \cdot k_b}{C_{OH}} \text{ und } C_{A''} = \frac{C_A \cdot k_s}{C_H} \quad \dots\dots\dots(21),$$

folgt nun direkt: 
$$\lambda = C_{A'} - C_{A''} = \frac{C_A \cdot k_b}{C_{OH}} - \frac{C_A \cdot k_s}{C_H} \quad \dots\dots\dots(22),$$

oder vereinfacht: 
$$\lambda = \frac{C_A - C_{A''}}{C_A} = \rho \left( \frac{k_b}{C_{OH}} - \frac{k_s}{C_H} \right) \quad \dots\dots\dots(23).$$

Substituieren wir nunmehr für  $\rho$  den Wert aus Gleichung (18), so ergibt sich:

$$\lambda = \frac{\frac{k_b \cdot C_H}{k_w} - \frac{k_s}{C_H}}{1 + \frac{k_b \cdot C_H}{C_w} \left( 1 + \frac{C_s}{k_s} \right) + \frac{k_s}{C_H} \left( 1 + \frac{C_y}{k_y} \right)} \quad \dots\dots\dots(24).$$

In dem Falle, dass echte Salzbildung vorliegt, würde sich der Ausdruck (24) vereinfachen zu:

$$\lambda_1 = \frac{\frac{k_b}{C_{OH}} - \frac{k_s}{C_H}}{1 + \frac{k_b}{C_{OH}} + \frac{k_s}{C_H}} \quad \dots\dots\dots(25).$$

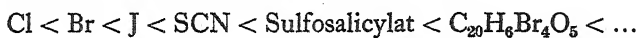


Indem nun im IEP definitionsgemäss der Wert  $\lambda = 0$  resultieren muss, lässt sich in gleicher Weise aus den Gleichungen (24) und (25) für die  $C_H$  beim IEP die bereits gegebene Gleichung (6) darstellen:

$$C_H \text{ bei } \rho_{\max.} = \sqrt{\frac{k_s}{k_b}} \cdot k_w \quad \dots\dots\dots(6),$$

d. h., dass der IEP (im Gegensatz zum  $\rho$ -Maximum) nicht durch die Salzbildung verschoben wird. Damit wird analytisch der Nachweis geführt, dass *der IEP nicht mit dem  $\rho$ -Maximum zusammenzufallen braucht*.

§ 9. *Lyotrope Einflüsse auf den Verlauf der  $\rho_{\max.}$ -Kurve.* Auch nach Salzzusatz zu Lösungen von Casein oder von hitzedenaturiertem Albumin tritt eine Verschiebung des  $\rho$ -Maximums derart ein, dass das Flockungsoptimum nicht mehr mit dem IEP koinzidiert (Michaelis<sup>(175)</sup>, mit Rona<sup>(182)</sup>, mit v. Szent-Györgyi<sup>(183)</sup>; s. auch<sup>(275)</sup> und Labes<sup>(134)</sup>!), und zwar erfolgt eine z. Tl. beträchtliche Verlagerung des Flockungsoptimums nach der weniger sauren Seite, sofern das Kation das wirksamere der beiden Ionen ist, oder nach der stärker sauren Flanke, wenn die Wirksamkeit des Anions überwiegt. Die Wirksamkeit der Ionen erstreckt sich dabei in erster Linie auf ihre *unterschiedliche Adsorbierbarkeit*. So ist für die Anionen z. Bsp. die einigermassen antivalente<sup>1</sup> Reihe



< und andere Anionen saurer Farbstoffe

konstatiiert worden, bei den Kationen eine Zunahme von den Erdalkali-, Alkali- und Schwermetallionen zu den Kationen der basischen Farbstoffe und Alkaloidsalze.

§ 10. *Die vier wichtigen Wendepunkte in der quasi isoelektrischen Zone.* In dem Bemühen von Michaelis, die Beziehungen zwischen den ionisierten Formen  $A'$  und  $A''$  und der neutralen Form  $A$  des Ampholyten durch die Dissoziationskonstanten  $k_s$  und  $k_b$  nach dem Massenwirkungsgesetze zu beschreiben, liegt auch die Voraussetzung (vgl. Pauli<sup>(205)</sup>, p. 188!), dass nur in einer  $C_H$  ausserhalb des IEP Salzbildung eintritt, indem der Säurezusatz anfangs nur die  $A'$ -Form zurückdrängt (Michaelis<sup>(171)</sup>, p. 251). Durch Untersuchung der Viskosität, der Alkoholfällbarkeit und des osmotischen Druckes ist am Serumalbumin und teilweise auch am Glutin gezeigt worden, dass das Maximum der Neutralteilchen nicht mit dem elektro-phoretisch gemessenen IEP zusammenfällt (Pauli<sup>(204)</sup>, pp. 35 sq.). Auch die Beobachtungen von Sørensen<sup>(263)</sup>,<sup>(265)</sup> an kristallisiertem Ovalbumin können unter Vergleich mit dem Kurvenverlauf der  $H^+$ -Aktivität eines solchen bei steigender Eiweisskonzentration nicht die frühere Auffassung stützen, dass Eiweisse bis zur Erreichung des IEP keine Säure binden (Pauli<sup>(205)</sup>, p. 193; vgl. ferner: Hasselbalch<sup>(82)</sup>, p. 129; Adolf und Spiegel<sup>(2)</sup>!). Durch Wo. Pauli<sup>(204)</sup> pp. 40 sq.) wird vielmehr gezeigt, dass beim Säurezusatz zu Eiweissen die verschiedene Valenz der vorhandenen negativen und der gebildeten positiven Eiweissionen mehrere Wendepunkte zur Folge hat, welche eine Übereinstimmung mit dem wahren, mittels

<sup>1</sup> Über die Aufstellung dieses Begriffes vgl. hauptsächlich: Schilow<sup>(255)</sup>, p. 427, Ostwald<sup>(202)</sup>, H. Pfeiffer<sup>(222)</sup>, p. 27)!

Reaktionsregulatoren ermittelten IEP vortauschen können, davon aber besser abgesondert bleiben. Allerdings rücken sie bei Verwendung von Moderatoren, welche die Ionisierungstendenz der benutzten Säure stark herabsetzen, wegen der verminderten Bildung polyvalenter positiver Eiweissionen einander so nahe, dass eine befriedigende Übereinstimmung erzielt werden kann. Von H. Pfeiffer<sup>(222)</sup>, p. 52) werden nach der Pauli'schen Beschreibung für die betr. Wendepunkte

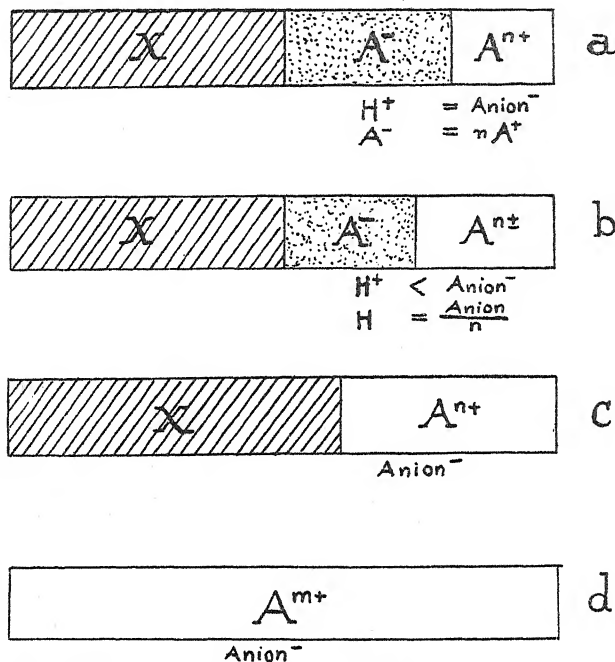


Fig. 5. Wo. Pauli's schematische Darstellung der vier Wendepunkte beim Zusatz von Säuren zu Eiweissen: (a) isoelektrisches Stadium, sobald  $\text{H}^+$  und Säurerestionen und ferner positive und negative Eiweissladungen an Zahl gleich geworden sind, schon in sehr niedrigen Säurekonzentrationen; (b) in Rücksicht auf den elektrophoretischen Transport *isomolares Stadium* bei starkem Überwiegen der negativen Säurerestionen gegenüber den positiven  $\text{H}^+$ ; (c) *Maximum der Neutralteilchen* und zugleich Optimum der Alkoholflockung im Bereiche kathodischer Elektrophorese (das Viskositätsminimum liegt nahe dabei, das Minimum des osmotischen Druckes bereits etwas vor Erreichen dieses III. Stadiums); (d) *Maximum der Säuresättigung* und der Ionisation (Bildung von Acidproteinen).

entsprechende Termini (*wahres isoelektrisches, isomolares oder quasi isoelektrisches Stadium*, ferner *Neutralteilchen- und Ionisationsmaximum*) geprägt (Fig. 5).

Die Verfahren zur Ermittlung des IEP beruhen nun nicht immer auf der direkten Aufsuchung jenes Stadiums im elektrophoretischen Experimente, sondern gründen sich oft auch auf die Ermittlung jener  $\text{C}_\text{H}$ , welche durch bestimmte Reaktionen ein Maximum der Neutralteilchen anzeigt. Auch die Methode Sørensen's liefert in diesem Sinne nur weitgehend angenäherte Resultate, wenn er auf der an sich richtigen Annahme, dass sich die Aktivität der  $\text{H}^+$  von reinem Ovalbumin mit dessen zunehmender Konzentration anfangs stark und später wenig ändert, jenen

Grenzwert aufsucht. Aus solcher Sachlage folgt, dass die Fixierungen des IEP wegen der verschiedenen Bestimmungsmethode nicht immer gleich gut vergleichbar sind. In demselben Sinne sind auch die weiteren Eigentümlichkeiten, die zu dem IEP von Zellproteinen in Beziehung gesetzt werden (Quellbarkeitsminimum, erhöhte Salzempfindlichkeit, Leistungsminimum der Zelle usw.; vgl. hierzu hauptsächlich: Weber<sup>(287)</sup>, H. Pfeiffer<sup>(216)</sup>, <sup>(217)</sup>, pp. 15 sq., Boas<sup>(21)</sup>, p. 23, u. a.!), jenem Wendepunkte nur angenähert eigentümlich. Eine weitere Komplizierung ergibt sich am protoplasmatischen Substrat dadurch, dass mit der Existenz je eines IEP für jedes Protein gerechnet werden muss (Reiss<sup>(241)</sup>, p. 28, H. Pfeiffer<sup>(222)</sup>, p. 56 und die dortige Literatur). Vermutlich resultiert ein allgemeiner IEP erst aus Kollektivwerten niederer Ordnung usw. Wohl kaum schon genügend wird von den Biologen auf die Ausbildung einer gewissen Breite (Zone) des isoelektrischen Verhaltens geachtet (vgl. aber z. Bsp.: Naylor<sup>(195)</sup>, Davidson<sup>(44)</sup>, Strugger<sup>(271)</sup>, p. 163!).

### C. DIE KOLLOIDCHEMISCHEN ERSCHEINUNGEN IN DER QUASI ISOELEKTRISCHEN ZONE.

#### (a) Das Ausbleiben elektrophoretischer Wanderung im IEP.

§ 11. Die Wanderungsrichtung als Funktion der Distanz gegen den IEP. Die Bestimmung des IEP nach der die elektrophoretische Wanderung aufhebenden  $C_H$  ist wohl nur selten an Geweben oder Zellen zu ermöglichen, aus technischen Gründen auch noch nicht versucht worden. Die Bedeutung dieser Bestimmungsweise als Standardmethode wird übrigens dadurch beeinträchtigt, dass nach experimentellen Befunden beim Eingreifen eines bestimmten Hydratationsgrades der Kolloidionen der Indifferenzpunkt der elektrophoretischen Bewegung auch nicht mehr streng mit dem IEP zusammenfallen kann (Bethe und Toropoff<sup>(15)</sup>, Loeb<sup>(142)</sup>, <sup>(145)</sup>, <sup>(146)</sup>, Gyemant<sup>(76)</sup>). Im übrigen ist der Wanderungssinn dargebotener Elektrolyte offenbar weitgehend von dem Verhalten der Zellkolloide entsprechend schwach negativ geladener Teilchen (H. Pfeiffer<sup>(218)</sup>, p. 454 und die dortige Literatur) gut zu erklären. Teilweise ist so die geförderte Aufnahme basischer Farbstoffe vor sauren zu verstehen (§ 20). Wegen der Abweichung der Plasmaeiwisse vom IEP wandert der Inhalt der Zellen embryonaler Wurzeln von *Pisum* zur positiven Elektrode (Meier<sup>(165)</sup>), wobei die Zellen kurz hinter dem Vegetationspunkte am deutlichsten reagieren, ältere und stark vakuolisierte dagegen nur sehr wenig (vgl. auch die Versuche an diversen Wurzelspitzen bei McClendon<sup>(164)</sup>!). Die sich zuweilen nach dem Wanderungssinne entgegengesetzt verhaltenden Chromatinpartikel werden als positiv geladen betrachtet. Die wahre Bewegungsgrösse als Mass der elektrischen Ladung, bestimmt nach der von Perrin<sup>(212)</sup>, <sup>(213)</sup> veränderten Helmholtz'schen Formel

$$u = \frac{\zeta \cdot E \cdot D}{4 \cdot \pi \cdot \eta} \dots\dots\dots (26)$$

(worin  $\zeta$  der Potentialsprung der Phasengrenzfläche,  $E$  das Potentialgefälle,  $D$  die Dielektrizitätskonstante der Flüssigkeit und  $\eta$  die Viskosität darstellt), kann freilich



nur in einer ganz bestimmten Kammertiefe korrekt gemessen werden (über die Durchführung siehe Svedberg<sup>(274)</sup>, v. Szent-Györgyi<sup>(276)</sup>, Putter<sup>(237)</sup>!). Die relative Grösse der Ladung von Blutkörperchen ist oft genügend zu beurteilen aus dem Vergleich gegenüber steigenden Konzentrationen eines Salzes mit dreiwertigem Kation (La-Nitrat). Als Mass der ursprünglichen (negativen) Ladung gilt dann diejenige  $\text{La}^{+++}$ -Konzentration, bei welcher die Elektrophorese zum Stillstand kommt (Kozawa<sup>(133)</sup>). Durch das elektrophoretische Experiment ist so eine unterschiedliche Ladung für Erythrocyten verschiedener Tiere konstatiert worden. Den Untersuchungen von Kozawa verdanken wir die Kenntnis der Reihe:

Lepus cuniculus > Cavia > Equus, Felis, Homo  
 Kaninchen > Meerschweinchen > Pferd, Katze, Mensch  
 > Canis > Capra > Ovis > Bos, Sus  
 > Hund > Ziege > Hammel > Rind, Schwein.

Von Coulter<sup>(40)</sup> bis<sup>(42)</sup> ist die Ladung so gemessen worden, dass ein Brei der Zellen in einem U-Rohr ein Diaphragma bildet, durch dessen capillare Spalten sich eine Flüssigkeit elektroosmotisch hindurchbewegen kann. Doch wird es sich bei vielen dieser und ähnlicher Bestimmungen nach unserer Ableitung um die Aufsuchung des  $\rho$ -Maximums handeln, welches im Gegensatz zum wahren IEP durch Salzwirkung eine Verschiebung erleidet (§§ 7–8), indem zufolge Haffner derart ausgeführte Versuche zeigen, dass die Lage des "IEP" auch durch Neutralsalze bestimmt wird.

(b) *Die Phänomene in der Nähe des  $\rho$ -Maximums.*

§ 12. *Das Minimum des osmotischen Druckes.* Dieses Minimum kommt in der Zone des Neutralteilchenmaximums dem wahren IEP noch am nächsten, liegt aber nach genauen Untersuchungen bereits im Bereiche kathodischer Wanderungsrichtung. Die direkte Messung der Wasseranziehung einer kolloiden Lösung als osmotischer Druck beruht teils auf der Zunahme der Moleküle in einem Proteinteilchen symbat einer Dispersitätsverminderung, teils auf der stärkeren Hydratation der Eiweissionen im Vergleich zu den unelektrischen Molekülen. Nach Loeb<sup>(144), (150), (152), (154), (158)</sup>, pp. 16, 88, 179 usw.) kommt die Wirkung von Elektrolyten auf den osmotischen Druck indessen nicht durch eine Veränderung des Dispersitätsgrades oder der Hydratation (oder überhaupt einer kolloidalen Eigenschaft) des Proteins zustande, sondern ergibt sich aus dem Konzentrationsüberschuss (kristalloider) Ionen *innerhalb* der Eiweisslösung über die äussere Ionenkonzentration. Die beobachteten osmotischen Drucke betreffen also z. Tl. auch den infolge eines Donnan-Gleichgewichtes auftretenden Druck, d. h. der gemessene Wert bedarf einer (sogen. Donnan-) Korrektur, welche sich nach Loeb zu  $2y + z - 2x$  bestimmt, wenn in molaren Konzentrationen bedeuten:  $y$  die  $\text{H}^+$  der freien Säure der Innenflüssigkeit,  $x$  die  $\text{H}^+$  der Aussenlösung und  $z$  die mit dem ionisierten Protein verbundenen Säurerestionen. Wegen der Errechenbarkeit der Donnan-Potentiale aus den Analysenwerten ist die Donnan'sche Theorie an dieser Stelle von Bjerrum angenommen worden. Loeb's Einwände gegen die Auffassung, dass

die osmotischen Drucke durch Dispersitätsverminderung entstehen, brauchen aber nicht unanfechtbar zu sein. Wenn das auch von Loeb (<sup>(141, (147) bis (149))</sup>) bestritten wird, so werden wir doch mit vielen andern Autoren daran festhalten können, dass beim Vergleich der Anionen nach dem Grade ihrer Verminderung des osmotischen Druckes in Eualbumin- u. a. Eiweisslösungen in neutraler Reaktion die Hofmeister'sche lyotrope Reihe entsteht (vgl. auch H. Pfeiffer (<sup>(222)</sup>), p. 26 sq.!).

Es ist nicht von vorn herein sicher, wie weit aus einer Senkung des osmotischen Druckes von Protoplasten auf eine Annäherung der sie aufbauenden Proteine an das  $\rho$ -Maximum geschlossen werden darf. Exakte Versuche zur Lösung dieser Frage liegen kaum schon vor. Zur Deutung zweier Maxima des osmotischen Druckes von Spermatozoiden (*Carcinus moenas*) bei pH 2.0 und 12.0 wird von Susaeta (<sup>(273)</sup>) ein Zusammenhang maximaler Ionisation der Zelleiweisse mit einer Dispersitätszunahme angenommen.

§ 13. *Das Viskositätsminimum.* Indem die Reibungskonstanten der elektrisch neutralen Moleküle kleiner als diejenigen der Ionen sind, zeigen Alanin, Glykokoll, Phenylalanin u. a. Aminosäuren im  $\rho$ -Maximum ein Viskositätsminimum. Auch die Reibungskonstanten der Anionen übertreffen noch diejenigen der Kationen und werden selbst übertroffen von jenen der Zwitterionen (Hedestrand (<sup>(84)</sup>)). Im Gegensatz zur Ionisation von Aminosäuren soll das Viskositätsverhalten von Gelatinelösungen gegenüber Säuren zum Donnan-Effekt in Beziehung stehen. Bei der Existenz submikroskopischer Proteinteilchen in genügender Quantität und in quellfähigem Zustande soll die Viskosität symbat dem osmotischen Drucke durch Elektrolyte verändert werden (Loeb (<sup>(153) bis (155)</sup>)). Wegen des übereinstimmenden Kurvenverlaufes der Viskosität und der Bjerrum'schen Ionenaktivität wird von Frisch, Pauli und Valkó (<sup>(205)</sup>, p. 187) gefordert, bei der Untersuchung der Viskosität der Säureproteine besonders die chemisch-konstitutionellen Änderungen der Proteinsalze zu beachten. Die Zunahme der Viskosität ist nach Pauli an die Anwesenheit der aktiven Ionen, die Abnahme an die fortschreitende Inaktivierung der Ionen geknüpft. Das bei dieser Wandlung durchlaufene Optimum wird von Loeb übersehen.

Von Biologen ist der Nachweis eines Viskositätsminimums noch kaum zu dem  $\rho$ -Maximum bzw. dem IEP in Beziehung gebracht worden. Das mag z. Tl. daran liegen, dass die zahlreichen bisherigen Untersuchungen über die Viskosität des Protoplasmas fast die Unmöglichkeit der Bestimmung von Durchschnittswerten gezeigt haben, vielmehr die Viskosität an demselben Objekt unter verschiedenen Bedingungen, von denen die Temperatur, die Belichtung; die Gravitation und die narkotische Lähmung noch die deutlichsten sind, äusserst variable Werte ergibt (vgl. Weber (<sup>(285), (288)</sup>), Boas (<sup>(21)</sup>), pp. 49 sq.!). Für die am besten bekannten Objekte kann bis soweit angeführt werden, dass eine Viskositätsverminderung einer Anregung der Plasmaströmung parallel geht, aber dem Leistungsvermögen des Protoplasmas entgegenläuft. Die in Kopulationsbereitschaft tretenden Zellen von *Spirogyra*-Fäden zeigen eine nach verschiedenen Methoden erwiesene Viskositätserhöhung (Weber (<sup>(286)</sup>), p. 282). Die bei der Befruchtung eintretende schnelle Variation des Viskositätsgrades (Chambers (<sup>(20)</sup>), Heilbrunn (<sup>(85)</sup>), Oedquist (<sup>(196)</sup>),

Seifriz<sup>(259)</sup>, Weber<sup>(286)</sup> ist von manchen Autoren (Boas<sup>(21)</sup>, p. 50, sowie Freundlich<sup>(59)</sup>, p. 295 und die dortige Literatur) auf Thixotropie zurückgeführt worden. Die ziemlich variable Viskosität des Blutes hängt wesentlich von der Zahl und dem Quellungszustande der vorhandenen Erythrocyten, aber auch von der Quellung der Plasmakolloide ab; den Zusammenhang zwischen diesen beiden Prozessen vermittelt wahrscheinlich der Aziditätsgrad (Schade<sup>(253)</sup>, p. 176).

§ 14. *Das Quellungs- (Imbibitions-) Minimum.* Wie die Viskosität zeigt auch die Quellung im  $p$ -Maximum ein Minimum. Beide Erscheinungen stimmen darin überein, dass das Dispersionsmittel z. Tl. durch Verankerung am Kolloid unter Bildung grösserer Aggregate in die disperse Phase geht. Daneben besteht demnach gleichzeitig eine enge Beziehung zum osmotischen Drucke.

Die nachweisbaren Analogien zwischen der Wasseraufnahme mancher Kolloide (Fibrin, Gelatine, Glutin, diverse Mehlproteine, Aleuron, usw.) und derjenigen bestimmter Gewebe (Muskel, Auge, Nervengewebe, usw.) berechtigen wohl zu dem Schlusse, dass die von den Zellen getragene Wassermenge normal und pathologisch von dem Zustande der Plasmakolloide bestimmt wird (Mart. H. Fischer<sup>(56)</sup>, pp. 128, 131, u. a.; s. auch<sup>(57)</sup>). Bei Behandlung von Geweben mit schwachen Säuren ist oft die Quellung eines Teiles der Zellkolloide mit einer Entquellung andere verbunden (Schade<sup>(253)</sup>). Mindestens teilweise muss diese Erscheinung mit der unterschiedlichen Lage des IEP (des  $p$ -Maximums) der wichtigeren Zellbestandteile erklärt werden. Sicherlich werden in der Zelle durch Salzeinflüsse sukzessiv mehrere Kolloide beeinflusst, so dass event. mehrere Quellungsminima nach Art des Höber'schen Beeinflussungsdiagramms resultieren (Boas<sup>(21)</sup>, p. 63; H. Pfeiffer<sup>(222)</sup>, p. 72). Den elektrischen Ladungsunterschieden der beiden Seiten lebender Membranen (Froschhaut) entspricht in gleicher Weise ein antagonistisches Quellungsvermögen und in Beziehung dazu die irreziproke Permeabilität (Wertheimer<sup>(289)</sup> bis <sup>(291)</sup>, <sup>(292)</sup>, p. 611 und die dort angeführte Literatur). Zur Erklärung der Quellung wird von Procter und Wilson<sup>(235)</sup>, <sup>(236)</sup> die Theorie der Donnan'schen Membrangleichgewichte<sup>(47)</sup><sup>1</sup> herangezogen. Danach ist die Kraft, welche den Eintritt von Wasser in das Gel verursacht und so die Quellung bedingt, der osmotische Druck der kristalloiden Ionen, die sich infolge eines Donnan-gleichgewichtes innerhalb des Gels in grösserer Konzentration als ausserhalb vorfinden; die Gegenkräfte, welche die Quellung schliesslich begrenzen, werden durch die Kohäsion zwischen den Kolloidteilchen gegeben. Loeb<sup>(151)</sup>, <sup>(154)</sup>, <sup>(158)</sup>, <sup>(159)</sup> hat sich dieser Auffassung angeschlossen. Von Pauli<sup>(205)</sup> werden Bedenken geäussert, nach welchen man dem an ein Eiweissalz anklingenden Substrat keine Membranfunktion zugestehen kann. Auch im Minimum ist immer noch eine so hohe Quellung nachweisbar, dass sie nach Paulis Schätzungen mit dem Donnan-Gleichgewichte nicht in Beziehung gebracht werden kann.

Der grösste Teil der Literatur beschäftigt sich naturgemäss kaum mit der Beziehung speziell isoelektrischen Verhaltens zum Quellungsgrade. Doch ist z. Bsp. der Nachweis eines Quellungsminimums in dieser Zone an Gelatine geführt

<sup>1</sup> Übrigens kann man durch elektrostatische Überlegungen zu dem gleichen Resultat gelangen [vgl. Wilson<sup>(295)</sup>].

worden (Chiari<sup>(30)</sup>). Wenn beim Kollagen die Bestimmung des  $\rho$ -Maximums als des Quellungsminimums nicht durchführbar ist, so dürfte dieser Befund mit der dafür angeführten Erklärung, jene Substanz sei eine *verschiedenartig* zusammengesetzte Mischung von Proteinen (Thomas und Kelly<sup>(278)</sup>), vorläufig gedeutet werden. Technisch kann man mit Pearsall und Ewing<sup>(208)</sup> die Quantität einer diffundierenden Substanz in Rücksicht auf die anfängliche Azidität bestimmen oder die Eigenschaften der Aussenflüssigkeit oder des Presssaftes untersuchen (Hempel<sup>(86)</sup>, Cohn<sup>(37)</sup>, Gustafson<sup>(75)</sup>) oder schliesslich die Imbibitionsfähigkeit der Gewebe vergleichen (Ulehla<sup>(280)</sup>). Von Pearsall<sup>(209)</sup>, <sup>(210)</sup> werden an Geweben von Solanum, Brassica, und Vicia Quellungsminima bei einer Aussenreaktion von pH 3,2 bzw. 4,5 bzw. 5,2–5,4 bzw. 6,2–6,5 gefunden, ohne dass man freilich schon angeben könnte, wie weit eine Verschiebung der intrazellulären Azidität durch die  $C_H$  des äusseren Mediums bewirkt wird (Hoagland<sup>(94)</sup> bis <sup>(96)</sup>, <sup>(98)</sup>, <sup>(100)</sup>, Crozier<sup>(43)</sup>, Osterhout<sup>(199)</sup>). Zur Erklärung nimmt Pearsall einen Rückgang der Permeabilität und des Vermögens der  $H_2O$ -Retention bei jenen Aziditätswerten an und weist zur Begründung auf die nachgewiesene<sup>(208)</sup> Abgabe von  $Cl'$  an das Medium hin. Der Permeabilitätsverlust soll durch Präzipitation oder Koagulation von Flasmakolloiden bewirkt werden (s. die Bemerkung bei H. Pfeiffer<sup>(222)</sup>, p. 75!). Der Temperatureinfluss auf das Quellungsverhalten, der auch von Stiles und Jørgensen<sup>(268)</sup>, <sup>(269)</sup> gefunden worden ist, wird so gedeutet, dass ein hitzebeständigeres Protein nach Fortfall der wegen grösserer Empfindlichkeit eher koagulierenden andern überwiegt und sich ein anderer "IEP" stärker auswirken kann. Möglicherweise liegt in der Hitzekoagulation eine gangbare Methode zum elektiven Nachweise bestimmter Proteine der Zelle in der von Pearsall gedachten Art vor (H. Pfeiffer<sup>(222)</sup>, p. 76). Lucke und McCutcheon<sup>(160)</sup> konstatieren an unbefruchteten Arbacia-Eiern eine annähernd konstante Quellung in Medien zwischen pH 5 und 9,8, hingegen ausserhalb der beiden Grenzpunkte eine starke Zunahme der Quellungsfähigkeit (s. auch Susaeta<sup>(273)</sup>!). Indem durch die extreme Azidität solcher Lösungen die Eizellen geschädigt werden, halten sie die erhöhte Quellung für eine nekrobiotische Erscheinung. Sicherlich gilt das aber nicht von dem Quellungsverhalten der Opuntia-Gewebe, welche Ulehla<sup>(280)</sup> p. 495 untersucht. Er findet eine Abhängigkeit des Quellungsgrades von der anfänglichen Azidität; daraus wird mit Recht geschlossen, dass man nicht (wie bisher zumeist) den Quellungseffekt im schliesslichen Gleichgewichtszustande (Imbibitionsmaximum), sondern den Imbibitionsgrad (die Imbibitionsgeschwindigkeit) zu vergleichen hat. Die Quellungskurve ist auch hier typisch zweigipfelig mit Maxima bei pH 3 und 10 und weist eine breite Zone minimaler Imbibition auf. Ulehla bezieht diese Verhältnisse auf den IEP (besser wohl: das  $\rho$ -Maximum) eines sich auf den Zelloberflächen befindlichen Ampholyten bzw. Ampholytengemisches.

§ 15. *Die minimale Lösungsstabilität (Flockung und Agglutination)*. Bei den von Wo. Ostwald sogen. isolabilen Eiweissen (Caseine, Euglobuline, Fibrine, manche denaturierte Eiweisse) erfolgt im  $\rho$ -Maximum eine Flockung ohne Zusatz; die stabilen Eiweisse (Glutin, Hämoglobin, manche Globuline, Albumine usw.) hingegen werden erst nach Zusatz von z. Bsp. Alkohol ausgeflockt, zeigen also in



diesem Aziditätsbereiche die grösste Alkoholempfindlichkeit (niedrigste "Alkoholzahl"; vgl. Pauli und Handovsky (206), (207), Schorr (256)!). Der bezeichnete Unterschied ist wohl chemisch damit zu erklären, dass im letzteren Falle die Ionen und die undissoziierten Moleküle eine gleich grosse Löslichkeit besitzen. Der gewöhnlich primär auf elektrische Entladung zurückgeführte Prozess resultiert nach experimentellen Erfahrungen an Ölgrenzflächen (Powis (225)) erst nach Unterschreitung eines kritischen oder Kontaktpotentials (über dessen Eigenschaften s. auch Bikerman (17)). Der Mechanismus soll nach der Theorie von v. Smoluchowski ((261); vgl. ferner: Zsigmondy (298), Westgreen (293), sowie: Michaelis und Davidsohn (178), (179), Brossa (27)!) darin bestehen, dass die Attraktionskräfte zwischen den Teilchen nach eingetretener Entladung (d. h. im  $\rho$ -Maximum oder in der Zone beim IEP) nicht mehr durch elektrostatische Abstossung ausgeglichen werden, so dass unter stärkerer Annäherung und Zusammenballung der Teilchen eine Trennung der dispersen Phase vom Dispersionsmittel bewirkt wird. Von Loeb ((140), (141), (147) bis (150), (157)) wird mit eintretender Flockung ein Minimum des Grenzflächenpotentials konstatiert, welches mit wechselnder  $C_H$  sowohl nach der sauren, als auch nach der basischen Seite bis zu einem Maximum zunimmt, dann aber rasch absinkt ((150), (151)). Durch Wo. Ostwald (201) wird für die Theorie der Elektrolyt-koagulation die Forderung nach einer stetig veränderlichen physikalisch-chemischen Grösse erhoben. Bestimmte Abweichungen von der theoretischen Valenzwirkung sind schon mit der ungleichen Adsorbierbarkeit isovalenter Ionen erklärt worden; doch befriedigt auch in dieser Form die rein elektrochemisch fundierte Deutung der Flockungserscheinungen durch Elektrolyte nicht.

Bemerkenswert ist die Abhängigkeit des Zusammenballens gewisser *Zellsuspensionen* von wechselnder elektrischer Ladung. Bei Bakterienaufschwemmungen wird der Beginn der Flockung durch ein Minimum des Kontaktpotentials angezeigt. Das Optimum der Säureagglutination der Bakterien ist nach neueren Erfahrungen (Eisenberg (49); vgl. auch Michaelis (170), Gillespie (73)!) nicht streng spezifisch. Bei der Hämagglutination (vgl. zu den Grunderscheinungen dabei z. Bsp.: Michaelis und Takahashi (184), v. Szent-Györgyi (277)!) wird die Zelloberfläche durch ein Agglutinin für die Elektrolyte angreifbar gemacht. Die Flockung von Blutkörperchen in isotonischen Lösungen von Nichteлектроlyten wird mit Säurebildung infolge Zersetzung (Radsma (238)) oder mit  $CO_2$ -Aufnahme aus der Luft erklärt. Die Lage des Flockungsoptimums der Goldsolreaktion auf den Liquor cerebrospinalis ist als eine Funktion der elektrischen Ladung des Liquor-Eiweisses erkannt worden. Das Flockungsoptimum bei normalem oder durch Tabes, multipler Sklerose und manchen Formen des Lues cerebri verändertem Liquor kann ins saure Gebiet (Meningitis) oder nach der alkalischen Seite (progressive Paralyse) verschoben sein (Bloch und Biberfeld (18)). Die Agglutinationshemmung durch Überschuss von  $H^+$  (Coulter (40), (42), Haffner (78)) oder geringe Neutralsalzkonzentration (Radsma (239)) ergibt eine etwas gestörte antivalente Anionenfolge, für Alkali- und Erdalkalikationen je eine besondere antivalente Reihe. Ein ähnliches Verhalten zeigen die Stromata der Blutkörperchen (Berczeller und Stanker (12)). Ähnlich verhalten sich auch pflanzliche Protoplasten aus der

Blattepidermis von *Tradescantia* (Kahho<sup>(113)</sup>). Für die Stabilitätsverhältnisse im Blute sind von fundamentaler Bedeutung der Gerinnungsvorgang (Stuber und Funck<sup>(272)</sup>) und die Erscheinung der bei Schwangerschaft und zahlreichen Krankheiten beschleunigten Blutkörperchensenkung. Entgegen der Höber'schen Theorie soll bei letzterem Vorgange die Stabilität der plasmatischen Eiweisse nicht eine Funktion der Lage des "IEP" sein (Wöhlisch<sup>(296)</sup>). Der gleichzeitig von Wöhlisch konstatierte Befund, dass beim Übergange des Fibrinogens in den Gelzustand eine Verlagerung des "IEP" nach der neutralen Seite zu stattfindet (Entladung des Fibrinogens) darf als Analogon zum Verhalten des Albumins bei der Denaturierung angesprochen werden. Den Grundgedanken der Höber'schen Theorie, nach welchem das labilisierende Moment z. Tl. die Entladung durch eine Eiweisschülle darstellt, kann man aber durchaus beibehalten. Dazu macht Wöhlisch die Annahme, dass das in den Erythrocytenhüllen adsorbierte Fibrinogen eine Denaturation zu Fibrin erfährt. So wird die der Hämagglutination parallel gehende Senkung sicher gut erklärt. Die aufgestellte Reihe der Blutkörperchen verschiedener Tiere bei der Säurehämolysen (*Cavia* > *Lepus cuniculus*, *Felis* > *Canis*, *Capra* > *Homo*, *Bos*, *Equus*, *Ovis* > *Sus*), bei welcher die Destruktion durch Quellungs- und Flockungsvorgänge gleichermassen bewirkt wird (Jodlbauer und Haffner<sup>(104)</sup>, <sup>(105)</sup>), entspricht einigermassen der Reihe für die Wärmehämolysen der Stromata (*Lepus cuniculus* > *Cavia* > *Felis* > *Equus* > *Homo* > *Ovis*, *Capra* > *Bos*), der Reihe für die Umladbarkeit und derjenigen für maximale Flockbarkeit in Essigsäureacetatpuffern. Die Annäherung an den durch Aziditätsveränderung gefundenen Wendepunkt in den Volumänderungen der Blutkörperchen ist verbunden mit erleichterter Flockung, während bei Entfernung von diesem Bereiche in das alkalische Gebiet eine zunehmende Quellung nebst Lyse der Stromata festgestellt wird, in höheren Konzentrationen wegen der Fällung des Hämoglobins aber die Übersichtlichkeit der Vorgänge gestört ist (Mond<sup>(192)</sup> und die dort angegebene Literatur).

Der Befund von Lepeschkin, nach welchem das Protoplasma von *Spirogyra* besonders leicht koaguliert, wenn verdünnte Lösungen schwacher Säuren zugegen sind, während die Erscheinung durch schwache Alkalien oder durch  $H_2O$  erschwert wird, ist von Pearsall und Ewing<sup>(209)</sup> p. 352 dahin gedeutet worden, dass jenes Plasma auf der alkalischen Seite des "IEP" eines bestimmten, durch Hitze koagulierbaren Proteins liegt (vgl. Cohn<sup>(35)</sup>, <sup>(36)</sup>). Innerhalb von Zellen kommt die Ausflockung wegen der überwiegend hydrophilen Natur des Protoplasmas im allgemeinen erst durch höhere Salzkonzentrationen zustande. Trotz der darin eingeschlossenen technischen Schwierigkeiten hat sich bei *Amoeba* mittels kleiner Mengen  $NaCl$  oder  $NaNO_3$  eine sehr fein dispergierte Fällung oder seltener die Bildung grösserer Granula nachweisen lassen (Giersberg<sup>(72)</sup>; vgl. auch Mills<sup>(185)</sup> und vor allem Strugger<sup>(270)</sup>, <sup>(271)</sup>!). Besonders leicht können Koagulationen im Karyoplasma oder in den äusseren Lamellen des Cytoplasmas bewirkt werden (Lepeschkin<sup>(136)</sup>, pp. 5 bis 44). Die Bildung von Bindegewebe-fasern, welche Nageotte<sup>(194)</sup> als das Ergebnis einer in der Zelle einsetzenden Fibrinkoagulation ansieht, ist jedoch wohl chemisch bedingt.

(c) *Das Aufsuchen des Azidifikationsgleichgewichtes (der Robbins-Effekt).*

§ 16. *Die Azidifikation des Mediums.* Nach der Definition verhält sich ein Ampholyt wie eine Säure, solange die  $C_H$  kleiner als diejenige seines IEP ist; im entgegengesetzten Falle gleicht der Ampholyt einer Base (§ 1), und nur im IEP wird der Zusatz des Ampholyten zu einer moderierenden Lösung deren Azidität nicht verändern (Michaelis (171); Sørensen (264), pp. 104, 192, u. a.). Unter Verwendung genügend schwacher und ausreichender moderierender Säuren ist also die Lage des IEP weitgehend von der Ampholytkonzentration unabhängig.

Es ist schon länger bekannt, dass pflanzliche Gewebe, die in moderierende Flüssigkeiten tauchen, deren  $C_H$  oft stark verändern und zu einem relativ konstanten Gleichgewichte meist im sauren Bereiche verschieben (*Azidifikation*).

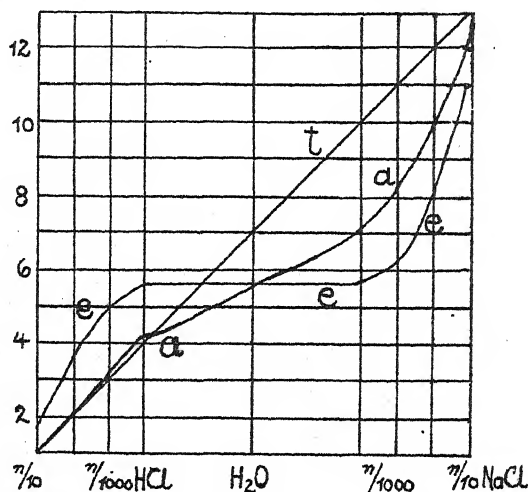


Fig. 6. Gleichgewichtskurve eines lebenden *Nachttriebes* von *Opuntia*. Auf der Abszisse sind die Säuren- und Basenkonzentrationen, auf der Ordinate die nach 48 Stunden erreichten Aziditätswerte der Versuchslösungen abgetragen. Die theoretisch zu erwartenden Werte ( $t$ ) folgen einer um  $45^\circ$  geneigten Geraden; durch Kontakt der Versuchslösungen mit der  $CO_2$  der Luft ergeben die regulierenden Gewebe die auf der  $a$ -Kurve gelegenen Werte; die  $e$ -Kurve schliesslich zeigt den Verlauf der Aziditätsveränderung in Vergleichslösungen ohne eintauchende Gewebeteile. (Nach VI. Ülehla.)

Für niedere Organismen ist diese Wirkung oft konstatiert worden; es sei nur an die Versuche von Boas und Leberle (22), Trautwein (279) und Cluzet nebst Mitarbeitern (33), (34) erinnert. Aber auch für höhere Pflanzen ist ein ähnliches Resultat nicht selten beobachtet worden (Arrhenius (3), Jones und Shive (106)). Aus neuerer Zeit seien dazu die Versuche von Rudolphi (249), (250), Robbins (242), (243) und Morea (193) erwähnt, und im übrigen sei auf die Literatursammlung verwiesen, die mit anderer Zielsetzung Mevius (167), pp. 113 sq. bietet. Diese Reaktionsregulation, die als Azidifikations- oder Robbins-Effekt (H. Pfeiffer (222), p. 82) bezeichnet werden möge, zeigen auch höhere Pflanzen ohne und mit erheblichen Schwankungen der intrazellulären Azidität (Fig. 6). Die Eigentümlichkeit ist bei lebenden Geweben an die Zelloberflächen gebunden (Ülehla (280), p. 484). Postmortal erstreben die Gewebe

einen andern Gleichgewichtswert. Zur Erklärung nimmt die Robbins'sche Schule an, dass aus der Aussenlösung hauptsächlich Anionen aufgenommen werden, bis im Azidifikationsgleichgewichte der Eintritt von Kat- und Anionen übereinstimmt (Robbins-Scott <sup>(245)</sup>, <sup>(258)</sup>). Durch das Abtöten erfolgt mit der Koagulation der Proteine eine Destruktion der Grenzflächen und zugleich die Ermöglichung der Ionenabgabe. H. Pfeiffer <sup>(222)</sup> vermisst dann aber eine Erklärung dafür, dass die Gewebe postmortal keinen scharf begrenzten IEP mehr besitzen. Hingegen will Denny zusammen mit Youden <sup>(297)</sup>, <sup>(46)</sup> die Azidifikation mit dem durch abweichende  $C_H$  der Gewebe bewirkten Austritt von Ionen in die (nicht immer auch moderierende) Versuchslösung deuten. Ihnen gelingt ein gleicher Effekt unter Verwendung künstlicher Mischungen organischer Säuren und Salze. Der Mechanismus soll in einer Präzipitation bestimmter organischer Säuren, in der Reduktion des organischen Radikals und in der geringeren Zurückdrängung der Säureionisation bestehen und so zu erhöhter  $C_H$  führen. In andern Versuchen, in denen eine Säurepräzipitation nicht angenommen werden kann, wird ein Salzeffekt infolge  $H^+$ - oder Moderatorenaktivität oder Komplexionisation angenommen. H. Pfeiffer <sup>(222)</sup>, p. 84) begründet die hervorstechende Bedeutung der Aktivität gerade der  $H^+$ . Neuerdings zeigt Úlehla <sup>(280)</sup> p. 501, wie Denny's Resultate und Deutungen mit denen der Robbins'schen Schule zu vereinigen sind. Danach steht dem von Robbins eingehend studierten *Regulationsmechanismus* (*Robbins-Effekt* nach unserer Bezeichnung), der die Existenz von Ampholyten voraussetzt und ganz in der von Robbins angenommenen Art wirksam ist, in den ausgetretenen Substanzen ein Regulations-schemismus gegenüber, welcher, wenn er von dem Mechanismus induziert worden ist, ungeachtet einer schon eingetretenen Regulation der  $C_H$  fortwirken kann. Als austretende Substanzen nennt Úlehla für die untersuchten Sukkulanten hauptsächlich  $CO_2$ ; doch können nach ihm bei andern Pflanzen (Rheum, Nymphaea) auch andere, meistens wohl moderierende Stoffe abgegeben werden.

#### D. WEITERE ALLGEMEINE BEZIEHUNGEN VON PROTOPLASTEN ZUR $C_H$

##### (a) Gruppierung der behandelten Fragen.

§ 17. *Rück- und Ausblick.* Durch die Betrachtung des Robbins-Effektes haben wir bereits die Überleitung zu denjenigen Eigentümlichkeiten von Protoplasten gefunden, die vielleicht nicht gerade *direkt* auf Reaktionen an Ampholyten (Proteinen) hinweisen, aber doch leicht mit der Annahme ihrer Anwesenheit und mit der Berechtigung der Unterscheidung eines IEP von Zellen ebenfalls in Beziehung gebracht werden können. Indem dabei die Stoffabgrenzung zuweilen Schwierigkeiten bereitet, wird zwar leicht eine gewisse Einseitigkeit in unsere Betrachtungen getragen. Doch haben sich schon Pearsall und Ewing <sup>(209)</sup> p. 352) dadurch nicht zurückschrecken lassen, auch diese Seite der Frage zu prüfen, und so dürfen wir ihnen auch bei ziemlicher Erweiterung der herangezogenen Befunde folgen. Es sollen nun in den folgenden §§ hauptsächlich die *Ionenaufnahme* und das *Leistungsvermögen* der Protoplasten in Beziehung zum IEP erörtert werden. Beide Themen werden uns Anlass geben zu mancherlei knappen Betrachtungen, die wir trotz



ihrer zuweilen noch erst unzureichenden Fundierung kurz andeuten wollen, auch wenn nicht alle die zu erwähnenden Erscheinungen die gesuchte Beziehung gleich gut erkennen lassen. Es scheint aber schon deshalb wertvoll, weil dadurch weitere Untersuchungen in der jeweils bestimmten Richtung angeregt werden. Schliesslich werden wir in diesem Hauptteile auch noch zu der Unterscheidung mehrerer koordinierter Wendepunkte an dem gleichen Protoplasten Stellung nehmen müssen, indem sonst von hier aus später ein Angriff auf die hier behandelte Arbeitshypothese erwartet werden könnte. Denn dem Bearbeiter scheint es schon heute sicher zu sein, dass auch dieser Abschnitt nirgends besser als in der Nähe der Behandlung der Zellenleistung abgehandelt werden kann.

(b) *Die Aufnahme von Ionen, insbesondere derer des  $H_2O$ .*

§ 18. *Die Ionenpermeabilität.* Es ist eine anerkannte Annahme der physikalischen Chemie, dass die Polarisierbarkeit von Phasengrenzflächen eine Funktion ihrer Ionendurchlässigkeit darstellt. Durch einen Wechsel der Ionenpermeabilität wird also auch im Protoplasma eine entsprechende Polarisierung an Grenzflächen hervorgerufen. Insbesondere bewirkt jede Störung der normalen Ionenmischung und so auch des Quotienten  $H'/OH'$  je nachdem eine Zu- oder Abnahme der Permeabilität (Bethe<sup>(13)</sup>,<sup>(14)</sup> und die dortige Literatur; Clowes und Smith<sup>(32)</sup>, Mevius<sup>(116)</sup>, H. Pfeiffer<sup>(218)</sup>,<sup>(220)</sup>, u. v. a.). Beispielsweise werden Blutkörperchen zwischen pH 8 und 8,3 für Anionen undurchlässig, dagegen für Kationen permeabel. Der Wendepunkt entspricht annähernd dem "IEP" des Globulins, auf dessen Anwesenheit in der Plasmahaut sich die Erklärung für diese Erscheinung gründet. Die Permeabilitätsänderung ist aber auch von andern kolloiden Zustandsänderungen weitgehend abhängig. Tritt z. Bsp. durch Wärme eine Koagulation ein, so ist damit eine Permeabilitätszunahme direkt verbunden. In diesem Sinne scheint auch die sichtbare Verdichtung der plasmatischen Aussenschicht nach Versuchen von Addoms<sup>(1)</sup> mit gesteigerter Permeabilität verknüpft zu sein. Sobald somit die plasmatischen Proteine durch die Azidität des Mediums dem IEP genähert werden, resultiert zugleich eine Permeabilitätsveränderung, die sich zumeist in der Abgabe oder Aufnahme von Ionen durch die Gewebe äussern muss (Robbins<sup>(242)</sup>,<sup>(244)</sup>). Versuche in dieser Richtung sind beschrieben worden über den  $Cl'$ -Austritt aus Zellen von Solanum-Geweben (Pearsall und Ewing<sup>(208)</sup>) und aus Nitella Zellen (Hoagland und Mitarbeiter<sup>(98)</sup> bis <sup>(100)</sup>), und Beziehungen ergeben sich von hier aus auch zu der Bethe'schen Reaktionsregel, die wir noch in andern Zusammenhänge (§ 20) betrachten werden (vgl. über die Permeabilitätsänderung durch irgend einen Aziditätswechsel auch: Harvey<sup>(81)</sup>, Snapper<sup>(262)</sup>, Haas<sup>(77)</sup>, Reemelin und Isaacs<sup>(240)</sup>, Collander<sup>(38)</sup>, Jacobi<sup>(102)</sup>, Haynes<sup>(83)</sup>, u. v. a. m.!).

§ 19. *Die Stoffaufnahme in das Protoplasma.* Mit der von der  $C_H$  abhängigen Ionenpermeabilität wird auch diese Erscheinung bestimmt. Zu den hier auftauchenden Fragen sei hauptsächlich auf Versuche von Davidson<sup>(44)</sup> verwiesen. Er schliesst aus der bevorzugten K-Aufnahme, dass bei einer Azidität bis zu pH 3,3 die meisten Kolloidampholyte elektronegativer als ihr IEP sind, sich also mit basischen Radikalen stärker als mit sauren beladen (§ 20). Mit veränderter Azidität

überschreiten bestimmte Kolloide jenen Wendepunkt und werden relativ elektropositiv, wie sich auch in wachsender P-Aufnahme äussert. Nach einer plötzlichen Aziditätsverschiebung des Mediums wird die P-Aufnahme stark erhöht, bis ein Gleichgewicht unter bevorzugter K-Absorption resultiert. So könnte also durch gleichzeitig auftretende elektropositive und -negative Ampholyte die selektive Aufnahme von Kat- und Anionen gewährleistet sein. Indem wir nur noch kurz zu dem noch völlig im Flusse der Tagesmeinung liegenden Gebiet der Stoffaufnahme in die Zelle auf die hier wichtigeren Versuche von Osterhout<sup>(198)</sup>, Hoagland<sup>(97)</sup> bis <sup>(99)</sup>, Lundegårdh und Morávek<sup>(163)</sup> und Philipson<sup>(223)</sup> hinweisen, dürfen wir als deren vorläufiges Ergebnis herausstellen, dass die selektive Stoffaufnahme nicht allein von der Konzentration des Nährmediums, sondern auch von dem gegenseitigen Mengenverhältnisse aller daran beteiligten Ionen abhängig ist. Wenn wir nun auch noch die Aziditätslage des Plasmas zum IEP seiner ampholyten Konstituenten zu beachten haben, so erhellt daraus einerseits die *Schwierigkeit der exakten Lösung* des Problems, andererseits die Erklärung für die manchmal unverständlich erschienene *Ungleichheit der bisherigen Ergebnisse*. Wie erst kürzlich exakt gezeigt worden ist (Hicks<sup>(92)</sup>,<sup>(93)</sup>), wechselt zudem die Stoffaufnahme im einzelnen mit dem Alter und besonderen Verhältnissen der Gewebe. Ob auch dann eine stofflich andere Zusammensetzung irgendwelcher Ampholytmischungen mit daher wechselnder Lage des IEP der Zelle ursächlich an den Phänomenen beteiligt ist, kann nicht entschieden, höchstens vermutet werden.

§ 20. *Das chemische Bindungsvermögen der plasmatischen Bestandteile* (vgl. Cohn<sup>(35)</sup>,<sup>(36)</sup>) ist wohl am bequemsten durch *Färbungsversuche* mit verschiedenen geladenen Farbstoffen darzustellen. Ohne in dem heutigen Bericht auf die Vitalfärbungstheorien näher einzugehen (s. darüber etwa: H. Pfeiffer<sup>(219)</sup>,<sup>(220)</sup> pp. 226 sq.!) sei doch auf Beziehungen der Färbbarkeit zum elektrochemisch bedingten Bindungsvermögen des Substrates hingewiesen. Die Voraussetzung zu einer Speicherung von Farbstoffen ist zwar auch durch das Aufnahmevermögen des Plasmas und durch die notwendige Möglichkeit des Ein- und Vordringens in die Plasmasschichten bedingt, mögen diese Beziehungen nun ausschliesslich durch Ruhlands Ultrafiltertheorie oder auch durch die wohl nur wenig bekannte und kaum schon genügend ausgewertete *Theorie des elektrocapillären Eindringens der Farbstoffe* (H. Pfeiffer<sup>(219)</sup>) gedeutet werden. Der Prozess der Farbstoffspeicherung wird dadurch aber noch nicht berührt. Die Grundidee der hier zu erwähnenden *Betheschen Reaktionstheorie*<sup>(13)</sup> deckt sich mit einer alten Erfahrung der technischen Färberei, nach welcher basische Farbstoffe von Textilgeweben in basischer Lösung am besten, weniger in neutraler oder gar in saurer Lösung gespeichert werden, während sich saure Farbstoffe gerade umgekehrt verhalten. Dasselbe scheint nach Erfahrungen an Gelatineplatten, geronnenem Eiweiss, Kollodiumhäuten und Agar auch von histologischen Präparaten zu gelten. Wenn nämlich auch die Färbbarkeit des lebenden Protoplasmas von der einen Seite lebhaft bestritten wird (A. Meyer<sup>(168)</sup>, Rumjantzew und Kedrowsky<sup>(251)</sup>), so ist doch von anderer Seite die Tatsache der Speicherung überzeugend sicher gestellt worden (Schaede<sup>(254)</sup>). Wenn aber auch die Speicherung saurer Farbstoffe im Innern der lebenden Masse erfolgt, so

wird sie durch v. Möllendorf (<sup>187</sup>), (<sup>191</sup>) nicht als Färbung präformierter Bestandteile des Plasmas betrachtet, sondern als eine "durch spezifische Eigenschaften der lebenden Cytoplasmen bewirkte extrem vitale Erscheinung." Hingegen soll die basische Granulafärbung mit der Ausfällung des Farbstoffes an der Oberfläche präformierter Teilchen eintreten (Herzfeld (<sup>90</sup>), v. Möllendorf (<sup>188</sup>), (<sup>191</sup>) p. 61). Durch diese Ausflockungsfärbung (vgl. auch H. Pfeiffer (<sup>221a</sup>), p. 431!) ermöglichen basische Farbstoffe im vitalen Versuche, aber auch im fixierten Präparate, die Darstellung besonderer, vermutlich saurer, Orte in den Geweben. Viele Autoren haben auch deswegen—ohne sich freilich auf basische Farbstoffe einseitig festzulegen, ja aus andern Gründen sogar gewöhnlich nur unter Verwendung von Säurefarbstoffen—die Methode der Vitalfärbung zum Messen der Zellazidität vorgeschlagen (vgl. hauptsächlich: Atkins (<sup>7</sup>) bis (<sup>9</sup>), Gillespie (<sup>74</sup>), H. Pfeiffer (<sup>214</sup>), (<sup>215</sup>), Small (<sup>260</sup>), (<sup>103</sup>)—weitere Literatur: H. Pfeiffer (<sup>218</sup>), p. 446). Wird auch durch v. Möllendorf (<sup>191</sup>) p. 66 die Färbbarkeit des lebenden Protoplasmas durch saure Farbstoffe zurückgewiesen, so treten doch weiterhin Bethe (<sup>14</sup>) u. a. für jene Bindungsmöglichkeit nach der Bethe'schen Reaktionsregel ein. Die Färbungsversuche von Rohde (<sup>247</sup>), (<sup>247a</sup>) an Pflanzenzellen und Geweben lebender Frösche, sowie die Befunde von Pohle (<sup>224</sup>) an Geweben des Hundes sprechen zugunsten dieser Auffassung, und Gellhorn (<sup>64</sup>) p. 606, und die dortige Literatur) findet sie in seinen Experimenten an Eiern und Spermatozoiden von *Holothuria* und *Strongylocentrotus* bestätigt. Trotz der bis in die neueste Zeit fortgesetzt erhobenen Einwände (Collander (<sup>39</sup>), Hertz (<sup>89</sup>), McCutcheon und Lucke (<sup>165</sup>), Schulten (<sup>257</sup>), Rumjantzew und Kedrowsky (<sup>251</sup>)) mag der vermittelnde Standpunkt nicht unberechtigt sein, dass neben der grundsätzlichen Richtigkeit des Bethe'schen Reaktionsprinzips dieses nicht geeignet zu sein braucht, um *alle* Probleme beim Vitalfärbungsversuche (insbesondere auch alle anzunehmenden Interferenzwirkungen) zu lösen. Auch die Untersuchungen von Irwin (<sup>108</sup>) bis (<sup>112</sup>) über den Eintritt und die Aufnahme von Farbstoffen Chloriden und organischen Substanzen in den Zellsaft hauptsächlich von Nitella zeigen nämlich, dass die Speicherung der Kationen (ausser H<sup>+</sup>) durch Lösungen von höherem pH-Werte gefördert wird. Eintretende Kationen können gleichzeitig eine OH<sup>-</sup>-Dissoziation bewirken, Anionen eine solche von H<sup>+</sup>. Daraus wird weiter gefolgert, dass (der Zutritt und) die Speicherung der Farbstoffe von vorhandenen Proteinen bedingt wird, welche sich zu beiden Seiten ihres IEP entgegengesetzt verhalten müssen. Den gleichen Schluss ziehen auch Robbins (<sup>242</sup>), sowie Pearsall und Ewing (<sup>209</sup>) p. 349).

§ 21. *Elektrohistologie und Elektropie*. Die elektrohistologische Methodik nach R. Keller (<sup>125</sup>) bis (<sup>128</sup>), (<sup>130</sup>); s. auch Kuwada (<sup>133 a</sup>!) und seinen Mitarbeitern Gicklhorn, Fürth und Blüh (<sup>65</sup>) bis (<sup>70</sup>), (<sup>62</sup>), (<sup>63</sup>)) beachtet ausser dem elektrisch bedingten Wanderungssinne wie v. Möllendorf (<sup>186</sup>), (<sup>190</sup>)) auch den Dispersitätsgrad derselben. Erstrebt wird die Auffindung intrazellulärer Potentiale. Eine Erschwerung der Versuche folgt aus der Negativierung der Zelle beim Anschneiden. Nach den Grössendimensionen sollen in den Zellen *Hochspannungselemente* vorliegen (<sup>131</sup>), (<sup>132</sup>)), weswegen von dem den natürlichen Verhältnissen weitgehend ange-

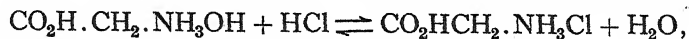
passten Hochspannungsmodell für elektrophoretische Versuche (Fürth<sup>(62)</sup>) Fortschritte erwartet werden. Befriedigend erklärt wird der physiologische Wandlungssinn von Farb- und Nahrungsstoffen, die gegen die Erfahrungen an Lösungen in destilliertem Wasser im Gewebe oft anodisch wandern. Wertvoll für die Annahme der Erklärung mit den Serulkolloiden als Farbstoffträgern sind die Kontrastpräparate mit verdünnten und konzentrierten wässrigen Lösungen basischer Farbstoffe.

Um die elektrostatischen Kräfte innerhalb von Zellen abzuschätzen, verwendet Karczag<sup>(115 bis 118)</sup> mit seinen Mitarbeitern<sup>(119 bis 124)</sup> Farbstoffe, in deren Molekülen die plasmatischen Ladungen elektrische Vorgänge induzieren, um dadurch selbst angezeigt zu werden. Die verwendeten elektropen Substanzen reagieren in wässrigen Lösungen mit einer Veränderung ihrer Konstitution (*Tautomerisation*). Diesem Prozesse der Elektropie unterliegen z. Bsp. gewisse (negativ geladene) Triphenylmethansulfosäurefarbstoffe (Fuchsin S, Lichtgrün, Wasserblau, usw.). Elektropisch untersucht worden sind Blutkörperchen<sup>(123)</sup>, Serum und Liquor cerebrospinalis<sup>(124)</sup>, sowie diverse Bakterien<sup>(119)</sup>. Bei den Vitalfärbungsversuchen<sup>(118, 120 bis 122)</sup>, durch welche Nervengewebe, quergestreifte Muskeln, Plattenepithel, sowie sekretorische Drüsen und lymphoides Gewebe als die am stärksten negativen Bestandteile des Tierkörpers erkannt werden, ergeben sich manche Berührungspunkte zu der Keller'schen Elektrohistologie. Wenngleich beide Arbeitshypothesen noch nicht zu einem Versuch, den IEP oder das  $\rho$ -Maximum zu den Ladungsunterschieden innerhalb der lebenden Zelle systematisch in Beziehung zu bringen, gelangt sind, scheint es doch zweckmässig, zur Anregung in dieser Hinsicht auch an dieser Stelle auf die beiden nicht überall ausreichend beachteten Methoden hinzuweisen.

§ 22. *Hydrolyse und Kondensation*. Die Hydrolyse, d. i. der aus der analytischen Chemie bekannte Prozess der Spaltung eines Salzes unter Beteiligung der Ionen des  $H_2O$  (Bjerrum<sup>(16)</sup>, Frary und Nietz<sup>(58)</sup>, Heyrovský<sup>(91)</sup>, Fricke<sup>(60)</sup>, Bogue<sup>(23)</sup>, Kailan<sup>(114)</sup> usw.), ist auch bei der Reaktion von Ampholyten zu beachten (Sørensen<sup>(264)</sup>, p. 151 u. a.), welche sich nach der Definition in saurem Medium wie schwache Basen, in alkalischer Umgebung wie schwache Säuren verhalten (§ 1). Der Hydrolyse von Salzen nach dem Schema



entspricht also ein ähnlicher Vorgang beispielsweise für Glykokoll und HCl in der Weise



oder vereinfacht:  $P + Q \rightleftharpoons R + H_2O \quad \dots\dots\dots(28),$

worin  $R$  das komplex gedachte Produkt darstellt, das z. Tl. hydrolytisch dissoziiert (hydrolysiert). Im Gleichgewichte stehen dann die betr. Konzentrationen  $C$ , wenn

$$\frac{C_R \cdot C_{H_2O}}{C_P \cdot C_Q} = K \quad \dots\dots\dots(29)$$

(oder im Bruche [Quotienten] Zähler und Nenner vertauscht) ist, wobei  $K$  eine Konstante darstellt (vgl. Bayliss<sup>(10)</sup>, p. 239!). Es wird also  $C_R$  am einfachsten



zunehmen können, wenn  $C_{H_2O}$  reduziert wird, d. h. die *Synthese von R erfolgt bei niedrigen  $C_{H_2O}$ -Werten*, und die *Hydrolyse ergibt sich umgekehrt aus erhöhten Werten*.

Es ist hier nicht der Ort, um über die durch eine Membran verstärkte Hydrolyse (*Membranhydrolyse*) ebenfalls abzuhandeln; dazu sei auf die Schriften zum Donnanpotential verwiesen.

§ 23. *Zur Fastigialtheorie*<sup>1</sup>. Der Befund, dass meristematische Zellen nur wenig vakuolisiert sind, differenziertes Zellmaterial dagegen während der Differenzierung immer mehr und grössere Vakuolen erhält, dass sich ferner meristematische Zellen von nicht wachsenden durch synthetisch (statt auf Kondensation) gerichtete Prozesse unterscheiden, wird von Pearsall und Priestley (<sup>211</sup>) p. 188 zur Grundlage einer Arbeitshypothese gemacht, die eine physiko-chemische Basis des auf Ent- und Redifferenzierung gerichteten Plasmageschehens liefern soll (Pearsall und Ewing (<sup>209</sup>), p. 355; vgl. auch H. Pfeiffer (<sup>220 a</sup>), p. 43; Priestley (<sup>227</sup>), (<sup>228</sup>)!). Unsere Kenntnisse der Proteinsynthese reichen freilich auch heute kaum schon aus, diese Analogie exakt zu begründen, und so können wir ebenfalls nur erst auf die ausgedehnten Versuche der Synthese von Kohlenhydraten und ihren Estern hinweisen, die eine allerdings überraschende Übereinstimmung erkennen lassen (Bourquelot und Bridel (<sup>24</sup>)). Von verschiedenen Seiten ist der Gedanke von Pearsall und Priestley aufgenommen worden (Weber (<sup>287</sup>); H. Pfeiffer (<sup>216</sup>), p. 79; (<sup>217</sup>) pp. 15 sq.; (<sup>221</sup>) pp. 167, 176; Vlès (<sup>282</sup>); Reiss (<sup>241</sup>), pp. 121 sq.). Die Auffassung ist von Priestley und Tupper Carey (<sup>232</sup>) inzwischen auch durch gegensätzliche Befunde zwischen Meristemen und wachsenden Zellgruppen belegt worden. Zusammen mit Woffenden (<sup>233</sup>), (<sup>234</sup>) versucht Priestley dann einen Einblick in die verschiedene Azidität der jederseits der Meristemzone gelegenen Zellen zu erlangen. Jedoch scheint die vorgeschlagene Methode, die den Anregungen von Atkins (<sup>9</sup>) nachgebildet worden ist, nach Untersuchungen von H. Pfeiffer (<sup>216</sup>) pp. 83 sq.) in manchen Fällen ungeeignet zu sein. Nach der neuesten Darstellung (Priestley (<sup>228</sup>), p. 16) besteht die von H. Pfeiffer als *Fastigialtheorie* bezeichnete Anschauung darin, dass die *Meristemzonen auf bestimmten Gradienten stärker und weniger saurer Reaktion* liegen (Fig. 7). Nach den soeben veröffentlichten Ergebnissen der nach Fürth (<sup>63</sup>) durchgeführten elektrometrischen Untersuchungen an Gewebekomplexen (und Einzelzellen) durch Gicklhorn und Umrath (<sup>71</sup>), durch welche gegenüber früheren Vorstössen in diese Materie (Ettisch und Péterfi (<sup>52</sup>), (<sup>53</sup>), Jost (<sup>107</sup>), Osterhout und Mitarbeiter (<sup>200</sup>)) ein bedeutsamer Fortschritt erzielt wird, zeigt sich u. a. das Xylem relativ positiv, das Phloem relativ negativ, wie ersteres aber auch Sclerenchym und in geringerem Grade Kollenchym, negativ ebenfalls das Mark und in wechselndem Masse das Rindenparenchym. Es ergibt sich also im ganzen eine Bestätigung der von R. Keller (<sup>125</sup> bis <sup>127</sup>)) nach der Färbbarkeit der Elemente erschlossenen Verteilung elektrischer Ladungen, welche auch die Voraussetzungen der Fastigialtheorie zu stützen geeignet wäre. Dass—aus technischen Gründen—die Cambiumzone durch Gicklhorn (<sup>71</sup>) nicht gemessen worden ist, bleibt für das hier erwünschte Ziel freilich bedauerlich. Es scheint auch nicht

<sup>1</sup> Abgeleitet von lat. "fastigium"—schräge Richtung nach unten (Abdachung) oder oben (Steigung).

sicher zu sein, dass man aus den angegebenen Befunden eine Bestätigung der Annahmen der Fastigialtheorie ablesen könnte. Im ganzen möchte vielmehr auch jene andere Deutung möglich sein, dass Gewebe mit teilweise sicher toten Zellen (Xylem, Sclerenchym, Fasern der sekundären Rinde) in auffallendem Gegensatz zu der Ladung allgemein lebender Zellen mit geringerer Positivität oder gewöhnlich ausgesprochener Negativität (Kollenchym, Parenchym, des Markes, des Phloems und der Rinde) stehen. Andererseits kann der Anhänger der Fastigialtheorie für sich geltend machen, wie die postulierten Aziditätsbeziehungen zwischen den die Meristemzone in Wurzel und Stengel flankierenden Gewebekomplexen vielfach gefunden worden sind (Priestley<sup>(227)</sup>). Auch dass z. Bsp. die Initialen der Seitenwurzeln innerhalb der Endodermis liegen, soll damit zusammenhängen (Priestley

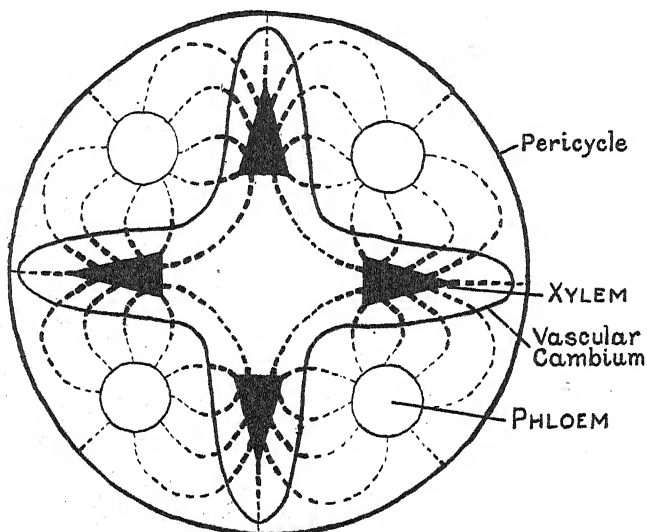


Fig. 7. Schematische Darstellung der  $C_H$ -Gradienten bei radialer Anordnung von Xylem und Phloem in pflanzlichen Wurzelachsen. Von den dreieckig gezeichneten Xylemzentren streichen abnehmende Gradienten unter Überschneidung der kreuzförmig verlaufenden Cambialzonen zu den kreisförmig angedeuteten Phloemkomplexen. (Nach J. H. Priestley.)

und Pearsall<sup>(231)</sup>). In gleicher Weise lassen sich auch die Befunde von Herklots<sup>(88)</sup> über die Wundheilung an Solanumknollen deuten, und in derselben Richtung liegen die Erklärungsversuche für die Lokalisierung von Korkbildungen überhaupt (Priestley<sup>(226), (230)</sup>). Bemerkenswert sind aber auch die von Fulmer<sup>(61)</sup> festgestellten Übereinstimmungen, über deren kausalen Zusammenhang freilich noch kein exakter Nachweis möglich gewesen ist. Danach liegt in einem synthetischen Medium mit  $NH_4$  das Wachstumsmaximum bei dem gleichen Verhältnis der Stoffe, bei welchem Gluten ein Quellungsminimum zeigt. Die Konzentration des  $NH_4$ -Salzes steigt beim Wachstumsmaximum von Saccharomyces und Quellungsminimum des Glutens gleichermassen mit der Temperatur, und die Erhöhung der Temperatur bei konstanter Konzentration des  $NH_4Cl$  ist der Herabsetzung der Salzkonzentration äquivalent.

(c) *Das Leistungsvermögen der Zelle in Beziehung zu ihrem IEP.*

§ 24. *Die Geschwindigkeit der Plasmaströmung.* Indem Strugger (<sup>270</sup>), (<sup>271</sup>) den Nachweis erbringt, dass zugleich mit gewissen morphologischen Veränderungen (Ausbildung der Vakuolen) des Protoplasmas durch  $H^+$  auch die Geschwindigkeit der Plasmaströmung beeinflusst wird, ist eine erste Grundlage für die Beurteilung dieser plasmatischen Leistung in Beziehung zur  $C_H$  und dem angenommenen IEP gegeben. Untersucht wird von ihm die immer zu beobachtende ("primäre") Strömung in Wurzelhaarzellen von *Hordeum*. Ohne hier entscheiden zu wollen, ob, wie Bělehrádek (<sup>11</sup>) will, jene primäre Strömung von traumatischen und photischen Reizen durchaus unabhängig ist, dürfen wir die von Strugger (<sup>271</sup>) gefundenen Minima im Neutralbereiche bei pH 6,85–6,90, sowie zwischen 7,00 und 7,05, schliesslich um ca. 7,35 hervorheben; daneben werden von ihm im sauren Gebiete noch zwei weitere Minima bei pH 6,4 und 5,85 angegeben (<sup>270</sup>). Der Verlauf des *Strömungsdiagramms* stimmt vollständig mit den Resultaten nach der Kurve der morphologischen Veränderungen überein, zeigt also gleichfalls vermutlich eine Beziehung zum IEP bzw.  $p$ -Maximum des Protoplasmas.

Dasselbe dürfte übrigens auch für die Abhängigkeit der *Teilung*, des *Wachstums* und der *Bewegung* von *Amoeba proteus* gelten, wenn wir an die Untersuchungsergebnisse von Hopkins (<sup>102</sup>) denken.

§ 25. *Das Plasmoptysen-Phänomen.* Die Plasmoptyse, d. i. das Hinausdringen von plasmatischen Massen infolge Platzens der Grenzschicht ("Ausspeien"), kann nach den oft zitierten Untersuchungen von Pantanelli (<sup>203</sup>) entweder osmotisch (Turgorspannung) oder durch Capillarspannung ("scopio-anosmotico") bedingt sein. Übrigens kann die Erscheinung gelegentlich auch für den betr. Organismus biologisch bedeutsam werden (Úlehla und Morávek (<sup>281</sup>), Demeter (<sup>45</sup>)). Gegenüber der Deutung bei Brinley (<sup>26</sup>), nach welcher das Platzen durch veränderte Permeabilität hervorgerufen werden soll, ist daran zu denken, dass bei seiner Versuchstechnik auch die Azidität des verwendeten Wassers die beobachtete Wirkung hervorgerufen haben könnte. Entgegen der Behauptung von Lloyd (<sup>138</sup>), dass vor dem Eintritt der Plasmoptyse kein Einfluss des betr. Mediums sichtbar sei, muss hervorgehoben werden, dass nach den Dunkelfeld-Untersuchungen Strugger's (<sup>271</sup>), welche die Beobachtungen von Úlehla und Morávek (<sup>281</sup>) bestätigen, vor der eigentlichen Explosion an der Spitze der Wurzelhaare ein sehr dünner Riss entsteht, aus dem etwas Plasma austritt (vgl. auch A. Fischer (<sup>55</sup>!)). Während der Eintritt einer Plasmoptyse in einer mit Flockungswirkungen am Plasma verbundenen  $C_H$  in solcher Weise eine geringe Zeit verzögert wird, erfolgt der Prozess in andern Medien, deren Azidität keine Flockung bewirkt, oft spontan. Nach den sehr sorgfältigen Untersuchungen von Strugger weicht das Plasmoptysendiagramm etwas von der Kurve der Ausfällung ab. Seine Optima liegen einerseits auf dem absteigenden Ast der Flockungskurve bei pH 7,0, andererseits im Flockungsminimum des schwach alkalischen Bereiches (ca. 7,18). Mit Recht weist auch er gleich Úlehla nebst Mitarbeitern (<sup>139</sup>), (<sup>281</sup>) darauf hin, dass bei der Entstehung von Plasmoptysen ausser den physiko-chemischen Wirkungen auf das Plasma auch der

Einfluss auf die Zellmembran beteiligt ist. Gegen die Auffassung von Stern<sup>(267)</sup>, der die Erscheinung mit negativen Osmosen verknüpft, kann man auch die Befunde anführen, dass Plasmoptysen in Acetatsgemischen hauptsächlich in den Flockungszonen, in Phosphatmedien dagegen meistens in dem Bereiche der Flockungsminima auftreten. Die wohl vorhandenen Beziehungen des Plasmoptysen-Phänomens zum IEP oder zum  $p$ -Maximum sind also sicher *durch andere Einflüsse* (ob neben dem *Wirkungsanteil der Membran* noch andere?) *überlagert*.

§ 26. *Tropistische Erscheinungen*. Wie auch manche Tropismen (*sensu lat.*) an Protoplasten mit dem Verhalten ihrer Proteinampholyte gegenüber der betr.  $C_H$  erklärt werden können, wird von Pearsall und Ewing<sup>(209)</sup> p. 353) an Beobachtungen von Spruit<sup>(266)</sup> gezeigt. Die Cilien von *Chlamydomonas* adhären nur in leicht saurem Medium an den Glaswänden, woraus geschlossen wird, dass ihr Plasmamaterial etwas nach der sauren Seite zu isoelektrisch ist, also in alkalischen Lösungen negativ geladen wird, so dass die Organe dann nicht am Glase haften.

Im allgemeinen scheint allerdings die mit den Tropismen sich beschäftigende Literatur noch keine Beziehung der Erscheinungen zum IEP der Proteine gesucht zu haben.

§ 27. *Das Leistungsoptimum des Protoplasmas* haben wir, wie am deutlichsten wohl von Boas<sup>(21)</sup> p. 23) ausgeführt worden ist, nicht im IEP, sondern in einer gewissen Entfernung davon zu suchen. Jener Grenzwert aber muss wegen der in seiner Nachbarschaft gefundenen geringen Stabilität der Kolloide das Minimum der Zellenleistung darstellen. Die vitalen Plasmageschehen sind in Hinblick auf die Gesamtleistung der Zelle dem zunehmenden Dispersitätsgrade und der zunehmenden Quellung und Hydratation gegenläufig, allerdings auch wieder durch eine Annäherung an den grobdispersen Bereich schliesslich begrenzt (vgl. hierzu Boas<sup>(19)</sup> und viele der hier nicht aufgeführten Versuche der Stoffwechselbeeinflussung durch quellende und entquellende Salzkonzentrationen). Namentlich bei Salzarmut, die entgegenwirkende sekundäre Salzeinflüsse ausschliesst, kann ein solcher Effekt durch eine *erhöhte Permeabilität* bewirkt werden. Dasselbe Ziel kann aber auch durch *Adsorptionsverdrängung* aus dem umgebenden Medium infolge unterschiedlichen Hydratationsbestrebens der anwesenden Ionen hervorgerufen werden, also event. trotz einer gewissen Entquellung, wie hauptsächlich Versuche mit Sulfaten lehren. Schliesslich kann auch eine *mässige Koagulation*, die nach einer von Traube, Winterstein, Lepeschkin und neuerdings von Süssenguth vertretenen Theorie mit einer Permeabilitätserhöhung verbunden ist, die gleiche Wirkung hervorrufen. Eine weitere Komplizierung ist endlich dadurch gegeben, dass ein bestimmtes Salz bei längerer Versuchsdauer einer gewissen Wirkungsänderung (*Umschlag der Wirkung*) unterliegen kann (vgl. Boas<sup>(20)</sup>, p. 29 u. v. a.!).

Es kann nun nicht verkannt werden, dass die Verbindung des IEP mit dem Minimum der Gesamtleistung des Protoplasten<sup>1</sup> nicht in wünschenswerter Weise die Gedanken der Fastigialtheorie (§ 23) zu stützen scheint. Doch kann immer noch angenommen werden, dass jene Arbeitshypothese nur eine *Annäherung an*

<sup>1</sup> Spezifische Leistungen, wie Wandbildung u. dgl., brauchen dadurch nicht unbedingt erfasst zu werden.



den IEP bzw. an das Maximum, nicht aber auch dessen Erreichung, zu verlangen braucht. In welcher Weise von hier aus auch eine Beziehung zu der von Růžicka als Entropieerscheinung gedeuteten *Plasmahysterese* beim Altern von Geweben gesucht werden kann, soll demnächst in anderm Zusammenhange und an anderer Stelle erörtert werden.

(d) *Das Auftreten mehrerer koordinierter Wendepunkte, die als IEP zu deuten sind.*

§ 28. *Das Zusammenfallen mehrerer  $\rho$ -Maxima.* Es ist schon einige Zeit aufgefallen, dass pflanzliche Wachstumskurven oft mehrgipfelig sind. Zwar haben Bryan<sup>(28)</sup> und Olsen<sup>(197)</sup> nur eingipfelige Kurven erhalten, doch werden von Arrhenius<sup>(4)</sup> bis<sup>(6)</sup>, sowie für das Wurzelwachstum durch Herčík<sup>(87)</sup> zwei Optima konstatiert. Ähnliches gilt auch für gewisse Pilze (Webb<sup>(283)</sup>, Lundegårdh<sup>(161)</sup>, Robbins<sup>(243)</sup>, Lindfors<sup>(137)</sup>). Ist auch in solchen Fällen die Deutung der Erscheinung mit der Annahme mehrerer "IEP" nicht immer versucht worden, so liegt eine solche Auffassung noch näher bei manchen andern Erscheinungen. Schon erwähnt worden sind die von Pearsall und Ewing<sup>(209), (210)</sup> erkannten Quellungsminima. So finden ferner Hitoshi Kihara (zitiert nach Strugger<sup>(271)</sup>, p. 164) am Pollenplasma dreigipfelige Kurven, Hopkins<sup>(102)</sup> bei der Abhängigkeit der Bewegung von *Amoeba proteus* von der  $C_H$  eine zweigipfelige Kurve, und bei der Abhängigkeit der Dickenzunahme der Gewebe von Meeresalgen von der Verdünnung werden von Lloyd und Ülehla<sup>(139)</sup> auxographisch zwei- und dreigipfelige Kurven konstatiert. Drei Minima des Längenwachstums von Wurzelhaaren sind auch bei Behandlung mit Medien verschiedener Azidität durch Farr<sup>(54)</sup> beobachtet worden, und schon früher haben Sakamura und Loo<sup>(252)</sup> unter Anwendung der Zentrifugierungsmethode am Plasma von *Spirogyra* für die Verfestigung oder Verflüssigung (den Viskositätsgrad) stets nur zwei- oder dreigipfelige Kurven gefunden. Die Dunkelfelduntersuchungen der Ausflockungsexperimente (Mikrosomenvermehrung) führen Strugger<sup>(270), (271)</sup> bei Beachtung des gesamten untersuchten Bereiches sogar zu 5 Wendepunkten, die nach dem Vorbilde der genannten Autoren mit dem "IEP" von Zellproteinen in Beziehung gesetzt werden. Die grosse Zahl der Kurvenenerhebungen gibt jedoch zugleich Anlass, ein wenig mit der vorzeitigen Verallgemeinerung einer solchen Erklärung zurückzuhalten. Es ist wichtig, dass Lundegårdh<sup>(162)</sup> an *Triticum*-Keimlingen durch Zusatz bestimmter Nährsalze zum Medium die zweigipfelige Kurve in eine eingipfelige umwandeln kann, und ähnlich erhält Webb<sup>(284)</sup> an Pilzsporen bei Benutzung verschiedener Nährmedien je nach deren Zusammensetzung eine verschiedene Anzahl von Kurvenenerhebungen. Unter Beachtung solcher *Interferenzwirkungen zwischen H' und den Ionen von Neutralsalzen* muss vielleicht bei den Strugger'schen Versuchen (ob auch bei bestimmten andern?) auch mit einem Einfluss der anderen Moderatorenzusammensetzung (im sauren Gebiete Essigsäureacetatgemische, im Neutralbereiche Phosphatlösungen) gerechnet werden. Eine andere Frage bleibt es allerdings, ob man dadurch die Kurve bis zur Eingipfeligkeit würde reduzieren können (?). Es bleibt also prinzipiell gegen seinen Erklärungsversuch nichts

einzuwenden, und es muss weiterhin mit der Häufung sich auswirkender Wendepunkte, die als IEP oder als  $\rho$ -Maximum ganz bestimmter im Protoplasma auftretender Proteïnampolyte anzusprechen sind, gerechnet werden.

#### E. DAS ERGEBNIS DER ANGESTELLTEN BETRACHTUNGEN.

##### § 29. Zusammenfassung.

(1) Der Begriff des IEP hat seine ursprüngliche Bedeutung auch in der physikalischen Chemie gegen eine neue eingetauscht.

(2) Die genaue Analyse zeigt die Existenz von vier Wendepunkten in der quasi isoelektrischen Zone: isoelektrisches und isomolares Stadium, Maximum der Neutralteilchen und der Ionisation.

(3) Nur der wahre IEP ist mit Stillstand der Elektrophorese verbunden. Die andern Wendepunkte, auch diejenigen im Maximum der Neutralteilchen (Minima des osmotischen Druckes, der Viskosität, der Quellung, der Lösungsstabilität), liegen bereits im Gebiete kathodischer Wanderung der Elektrolytteilchen.

(4) Auch in der biologischen Literatur wird der Begriff des IEP für das Maximum der Kurve des Dissoziationsrestes ( $\rho$ ) verwendet. Es besteht jedoch ein Unterschied zwischen diesen beiden Wendepunkten, indem der IEP im Gegensatz zum  $\rho$ -Maximum durch Salzbildung nicht verschoben wird.

(5) Zellen und Gewebe von Pflanzen—und vielleicht auch von Tieren—zeigen viele Reaktionen in derselben Weise wie Ampholyten mit einem IEP (Imbibitionsfähigkeit, Verhalten gegen Flockungsmittel, Azidifikation eines Mediums, chemisches Bindungsvermögen usw., wahrscheinlich auch Permeabilität und Adsorption von Ionen).

(6) Wahrscheinlich wird diese Erscheinung hervorgerufen durch die Anwesenheit von Ampholyten auf den Oberflächen der Zellen und an ihren Grenzflächen im Innern des Protoplasmas.

(7) Das Ampholytverhalten von Geweben und die Realität ihres IEP ist allerdings noch zu wenig geprüft worden in Hinblick: auf die Ionenpermeabilität und Stoffaufnahme durch das Protoplasma, auf das elektrohistologische und elektropische Verhalten von Zellen, auf die Hydrolysen und Kondensationen im Protoplasma, auf den Mechanismus der Einwirkung auf bestimmte physiologische Leistungen der Zelle, wie Plasmaströmung u. dgl.

(8) Bei der Azidifikation gibt es neben dem Regulationsmechanismus (sogen. Robbins-Effekt) gegenüber dem Medium auch einen Regulationsschemismus (= Schematismus), der von jenem induziert wird.

(9) Es ist nicht zu verlangen, dass die Charakterisierung der Gewebe als Ampholyte (d. h. die Annahme eines IEP von Zellen oder Geweben) sämtliche hier erwähnten und die damit zusammenhängenden Fragen endgültig lösen wird. Aus diesem Grunde braucht auch die Bethe'sche Reaktionsregel nicht vollkommen zu befriedigen (cf. auch § 25, Plasmoptysis).

(10) Die Hypothese von der Realität eines IEP von Zellen und Geweben stützt u. a. die Fastigialtheorie zur physikochemischen Erklärung der Meristematisierung (Embryonalisierung, Entdifferenzierung) von (pflanzlichem) Zellmaterial.

(11) Im IEP der Plasmaproteine finden wir zugleich ein Minimum der physiologischen Zellenleistung.

(12) Das Vorkommen verschiedener Proteine im Protoplasma bewirkt nicht stets einen kollektiven IEP, sondern in manchen Fällen sind nach den heutigen Auffassungen verschiedene IEP in bestimmten Aziditätsbereichen von überwiegender Bedeutung (sukzessives Ausscheiden der andern?).

#### § 29. Summary.

(1) The conception of the IEP has altered its original meaning both in biology and in physical chemistry.

(2) Exact analysis shows the existence of four turning-points in the quasi-isoelectric zone: the isoelectric and isomolar stages, and the maxima of neutral particles and of ionisation.

(3) The true IEP alone is connected with the stationary phase of electrophoresis. The remaining turning-points, including those at the maximum of neutral particles (minima of osmotic pressure, of viscosity, of imbibition, of solution stability) lie within the region of the cathodic migration of electrolyte particles.

(4) In biological literature the conception of the IEP is employed for the maximum point of the dissociation curve ( $\rho$ ). Nevertheless there is a difference between these two turning-points in that the IEP, but not the  $\rho$ -maximum, is not displaced by salt formation.

(5) Cells and tissues of plants, and perhaps also of animals, show many reactions similar to those of ampholytes with an isoelectric point. Such are the capacity of imbibition, behaviour towards flocculating agents, acidification of the medium, chemical affinities, etc., and probably also permeability and adsorption of ions.

(6) Probably this phenomenon is due to the presence of ampholytes on the surfaces of cells and at the boundaries in the interior of the protoplasm.

(7) The ampholyte behaviour of tissues, however, and the reality of their IEP has up to now been insufficiently tested from the points of view of the permeability to ions and the uptake of substances by protoplasm, of the electrohistological and electropic behaviour of cells, of hydrolyses and condensations in protoplasm, and of the mechanism of particular physiological cell functions such as protoplasmic streaming and so on.

(8) In the acidification phenomenon there exists, in addition to the regulatory mechanism (so-called Robbins effect) towards the medium, also a chemical regulation (schematism) induced by this.

(9) It is not assumed that by regarding tissues as ampholytes (*i.e.* by the assumption of an IEP for cells and tissues) all problems will be finally settled. For this reason Bethe's law of reaction cannot be expected to be completely satisfactory (*cf.* also § 25).

(10) The hypothesis of the existence of an IEP of cells and tissues supports among others the fastigial theory which gives a physico-chemical explanation of meristem formation (dedifferentiation) in plant tissues.

(11) At the IEP of plasma proteins there is a minimum of physiological functions.

(12) The presence of different proteins in protoplasm does not always bring about a collective IEP, for in many instances, according to present conceptions, certain IEP's in definite regions of acidity are of predominant importance, possibly in consequence of the successive elimination of the others.

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# THE DERMATOPHYTES OR RINGWORM FUNGI

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(Received September 11, 1928.)

(With Seven Plates.)

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## 1. INTRODUCTION AND HISTORICAL.

### I. INTRODUCTION.

DISEASES of man and animals caused by fungi are known as "Mycoses." There are many different kinds of mycoses caused by different fungi, and the same fungus may cause clinically distinct mycoses when it attacks different organs or tissues. The different mycoses may be distinguished either by combining the generic name of the causal fungus with the suffix "-osis," as in *Aspergillosis* and *Trichophytosis*; or by prefixing the name of the organ or tissue attacked to the term mycosis, as in *Pneumomycosis*. In the latter terminology fungous diseases of the skin are called Dermatomycoses. The popular term "ringworm" is nearly synonymous with dermatomycoses; but there is this difference, that "ringworms" are generally considered to be affections which are entirely superficial and never invade tissues underlying the dermis, while dermatomycoses include all these affections, and, in addition, skin diseases which involve deeper-lying tissues. It is convenient to have a general term to cover all the fungi which cause dermatomycoses, and the name Dermatophytes is applied to them. The name Dermatophytes is usually employed in a restricted sense, and is applied, not to all the fungi causing dermatomycoses, but only

to those which cause superficial skin diseases or ringworms. It is in this sense that the term Dermatophytes is used in the present work. It should be noted that the term is purely one of convenience, and in itself does not imply that there is any relationship between the numerous species of fungi to which it refers.

The medical term for ringworm is *Tinea*, and the different types of ringworm are distinguished as *Tinea tonsurans*, or scalp ringworm; *Tinea circinata*, or ringworm of the glabrous skin (sometimes called *Herpes circinata*), and so on. A few of the other common medical terms for special types of ringworm may be mentioned. Mycotic sycosis is the name given to ringworm of the beard, especially when it is associated with suppurative lesions. Kérion, or Kérion of Celsus, is the term applied to ringworms which are more deeply seated than the ordinary forms and are more or less suppurative. Ringworm fungi frequently affect the nails of the fingers or toes, and such affections are called Onchomycoses. Finally, Favus is a special form of ringworm in which bright yellow crusts, formed of numerous very small cup-like scales, are developed on the lesions, which have a characteristic odour, usually described as mouse-like.

## II. HISTORICAL.

*Discovery of parasitic nature of ringworm.* According to Sabouraud (1910), Remak, in 1837, was the first to observe that the scutula of favus are aggregations of fungous hyphae, but he failed to realise that the fungus was the cause of the disease. The fungous nature of the "dry pustules" of favus was demonstrated in 1839 by Schönlein. For a long time opinions were divided as to the importance of the fungus, some maintained the causal significance of the fungus, while others considered that it was only an accidental growth on the purulent secretion.

These discoveries were scarcely known outside Germany when, in 1841, Gruby, working in Paris, published a paper entitled "Sur une végétation qui constitue la vraie teigne" in the *C.R. Acad. d. Sci.* (cited in full in Sabouraud, 1910), in which a remarkably precise and detailed account of the fungus causing favus is given. This account was neither confirmed nor added to for nearly 30 years. Lebert, in 1845, named the fungus *Oidium schönleinii*, but, in the same year, Remak founded the genus *Achorion* for it and it was definitely named *Achorion schönleinii*. Gruby published three further papers (all cited in full in Sabouraud, 1910) in the succeeding years, the four papers together showing the mycotic origin of all types of human ringworm. In as far as they deal with the fungus, these papers are excellent, but they are very poor as regards the clinical descriptions and in some cases the condition described is wrongly named. The second paper, published in 1842, describes a fungous parasite of the type later called *Trichophyton ectothrix* by Sabouraud. In the third paper he described the parasite of "small-spored ringworm" or "microsporosis" and named it *Microsporum audouini*. His final paper, 1844, describes with remarkable accuracy the parasite of "tinea tonsurans," which belongs to the type later called *Trichophyton endothrix* by Sabouraud.

In these four short papers Gruby described very well the morphology of the parasite, its development, and the invasion of the hair, for the four distinct types

of ringworm diseases common in man: favus; ringworm due to ectothrix Trichophyton; small-spored ringworm due to Microsporum; and large-spored ringworm due to endothrix Trichophyton.

In 1845 Malmsten created the generic name *Trichophyton* for the parasite of ringworm. Once the parasitism of the fungi in ringworm was demonstrated, little important work materialised until Sabouraud began his investigations in 1892, and succeeded, in a few years, in clearly showing the remarkable "plurality" of the ringworm fungi. By "plurality" is meant that the different types of ringworm are not due to the same parasite, but that each clinical type may be caused by one of a group of fungi which differ among themselves, and, more markedly, from those found in other types of ringworm.

The first recognition of suppurative ringworm, now known to be caused by pyogenic Trichophytons originating from animals, notably the group represented by *Trichophyton asteroides*, was in 1856, when Tilbury Fox showed the trichophytic origin of Kérion. He, however, considered it to be a trichophytosis complicated by inflammatory lesions due to secondary infection by bacteria. This opinion prevailed generally until 1893, and is still maintained by certain dermatologists.

*Animal ringworm.* Ringworm of animals was first recognised in 1853 in Paris by Bauley and Raynal, whose observations were described by Bazin (Sabouraud, 1910). The parasite was seen to infiltrate the hair as in man, but it was noticed that the spores were smaller. Gerlach, in 1857-9, performed systematic experiments on the transmission of ringworm from animal to animal; and Raynal carried out experiments of a similar nature and demonstrated the transmission to man of ringworm from horse and cow. Mégnin, in 1878, from a clinical study of horse ringworm concluded that there were two species of animal Trichophytons. He tried to prove this by inoculating a dog with two different ringworms at the same time, one from a horse and one from a cow; and he found that the resulting lesions were of two distinct clinical types.

*Cultivation of ringworm fungi.* As early as 1845, Remak attempted to cultivate *Achorion schönleinii*, and succeeded in obtaining some growth on apple, but within a few days it was overgrown with *Penicillium*. Gruby also attempted to cultivate the fungi and claimed to have obtained growth on wood.

The general opinion at first was that the ringworm fungi were simply ordinary saprophytic fungi, and were special stages of forms like *Penicillium*. The constant appearance of ordinary saprophytes, on the substances on which cultivation was attempted, fostered this conception; but the crucial test of inoculation was adopted by Kobner in 1864, and thus settled the matter, as Trichophytons produced the characteristic lesions while the common saprophytes did not.

In Sabouraud's opinion the first pure cultures of ringworm fungi were obtained by Grawitz, who in 1886 obtained cultures of *Trichophyton* and *Achorion*. The two cultures were very different and inoculation with them produced the corresponding diseases. Ten days later Duclaux also obtained cultures of these fungi, and obtained similar results to those of Grawitz.

Nearly two years later Verujsky (1887), a pupil of Duclaux, published a

comparative study of *Trichophyton* and *Achorion*. In addition to useful morphological facts, the paper dealt with the biology or physiology of the two fungi comparatively, and showed striking differences between them. This paper definitely established the differentiation of *Trichophyton* and *Achorion*, and was the first definite proof of plurality in the ringworms.

The next advance was made by Kral in 1890, when he obtained cultures from a case of favus which differed from the ordinary *Achorion*, and thus established the plurality of the *Achorions*.

In the same year Mégnin, who as previously mentioned maintained the plurality of the *Trichophytons* on clinical grounds, presented at the Société de Biologie two distinct cultures corresponding to his clinical types; and the following year he presented another culture which was isolated from a fowl and coloured the medium a raspberry-red colour, this fact clearly indicating that it was a culture of the parasite later known as *Achorion gallinae*.

*Plurality of ringworm fungi.* To a limited extent the plurality of the Dermatophytes had now been established, as it was recognised that *Trichophyton* was distinct from *Achorion* and that both represented two or more species. As the specific differentiation of the Dermatophytes depends to a great extent on the characteristic type of cultures which are formed on certain solid media, the demonstration of the large number of different species which occur awaited the adoption of such methods of cultivation.

It was at this stage that Sabouraud began his research at the instigation of Besnier, and knowing nothing of any previous work on the subject, save that of Verujsky. He adopted a threefold line of attack, modelled largely on the methods current in bacteriological research. His plan of work was to make a careful clinical study of each case and to keep full notes of the clinical aspect; to examine the hairs microscopically and keep permanent preparations of them for comparison; and to make cultures from every case, and to keep them for comparison with those from other cases. By these methods it at once became apparent that there were two types of child ringworm, which may be distinguished by the naked eye, microscopically, and by cultures, these being "small-spored ringworm" and "large-spored ringworm."

By introducing a series of suitable solid artificial media of fairly constant composition, composed of sugar (maltose, glucose, mannite and lactose) 3.7 per cent., peptone 1 per cent., and sufficient agar for solidification; and making series of cultures from a number of cases on the same series of media at the same time, he was able to show that his two parasitic types were not single species, but groups of species. To make sure that the different species retained their specific characters, which consisted in the form of the cultures developed on the various media, they were sub-cultured side by side on similar media for several generations. Of 54 cultures of large-spored ringworms, this method revealed that there were no less than 19 distinct species.

His next paper dealt with the pyogenic or suppurative ringworms, and showed that they formed a homogeneous group of six species. In some of these the animal

origin was known, and it was regarded as probable for all of them. These forms are the ectothrix Trichophytions.

Next he dealt with the ringworms of the beard, and showed that, in France, such ringworms are not generally caused by the common parasites of child ringworm, but are usually due to ectothrix Trichophytions.

At this stage he first became acquainted with Gruby's work, a study of which induced him to investigate small-spored ringworm caused by *Microsporum*.

The idea of the plurality of the Microsporums made slower progress than in the case of *Trichophyton* and *Achorion*. Some progress was made between 1892 and 1894, when Sabouraud described a rapidly growing *Microsporum*, once on man and four times on a horse, which was probably *M. lanosum*. In 1897 Bodin described *M. canis* on dog; and Fox and Blaxall and Adamson described a *Microsporum* of the cat. Later work by Bodin, Mibelli, Minne, Uriburu, Suis, Zallikoper, and Sabouraud established a remarkable degree of plurality on this group. The chief result of this work was to show that there are two groups of Microsporums, one with small or medium-sized cultures, and the other with large luxuriant cultures. The latter are of animal origin, and it is among them that the greatest variety is found; and the forms with small cultures are purely human ones, and are relatively few in number.

*General progress after 1894.* Later work has been, on the whole, simply confirmation or extension of Sabouraud's results, and little of great interest or importance has come to light.

In England Adamson (1895, etc.) was one of the first to verify the clinical, microscopical and cultural characteristics of small-spored ringworm. Fox and Blaxall (1896-7, etc.) worked with large numbers of cultures and confirmed Sabouraud's work in a general manner.

Little was added to the knowledge of the plurality of the Trichophytions after Sabouraud's work. Most workers admitted the principle of plurality, but considered that Sabouraud had carried it too far and had split up some groups into an unnecessarily large number of species. His answer to this criticism is that he worked with many hundreds of cases and cultures, while his critics handled comparatively small numbers, and were consequently not in a position to meet with as many species as he did.

Fox (1897) insisted on the relationship of *Microsporum* and *Trichophyton*, which Sabouraud had considered to belong to different families. Later Sabouraud came to the same conclusion as Fox, and, in 1900, added *Achorion* to the same family as *Microsporum* and *Trichophyton*. About this time Matruchot and Dassonville (1899) studied the relationship of the Dermatophytes to other fungi and concluded that the three genera, *Microsporum*, *Trichophyton* and *Achorion*, could be placed among the Gymnoascaceae.

Finally in 1910 Sabouraud, in his large work entitled *Les Teignes*, brought together all his own researches and embodied them in a general work containing the substance of previous work on the subject up to that date. This epitome of the knowledge of the Dermatophytes forms the starting-point for all later work, and



may be considered as marking the end of the history of the subject, and the beginning of current research.

## 2. MORPHOLOGY AND DEVELOPMENT.

### I. PARASITIC LIFE.

*Introduction.* As this work is concerned with the botanical aspect of the fungi causing ringworm, it would be quite out of place to enter into discussion or description of the pathology of the diseases. As the fungi dealt with may be regarded as being primarily parasites of the hair, although strictly speaking this is not true of all Dermatophytes, it will be sufficient to give a brief account of the behaviour of the main types of fungi during their parasitic life on the hair, without entering into details about the differences in the clinical conditions thereby occasioned. In the broad sense of the term, ringworms may be divided into four main types: small-spored ringworms due to *Microsporum*; small-spored ringworm due to *Trichophyton*; large-spored ringworm due to *Trichophyton*; and favus, which is a widely different type and is due to *Achorion*.

*Microsporum.* The distinguishing feature of ringworm due to species of *Microsporum* is that the basal portion of infected hairs is surrounded by a sheath of closely aggregated, very minute spores, which extends a few millimetres beyond the follicle, and is easily visible to the naked eye as a whitish coating around the lower part of extracted hairs.

Adamson (July, 1895); Fox and Blaxall (July, 1896); and Sabouraud (1910) have worked out the mode of infection of the hairs and the growth of the fungi therein. Neglecting minor differences of opinion on less essential points, the general behaviour of a *Microsporum* from the time of infection is as follows. The initial infection is always epidermal, where the fungus appears as a network of fine, sparsely septate, sinuous hyphae in the epidermal scales. When the hyphae reach the mouth of a hair follicle they emerge from the epidermis and proceed to grow downwards into the follicle, between the wall of the follicle and the hair. Considerable proliferation of the hyphae occurs at the mouth of the follicle, with the result that a cone-shaped mass of aggregated chains of large rectangular cells and large oval cells is formed. From this hyphal mass large, "giant," septate hyphae descend along the surface of the hair towards the root. The ultimate fate of the giant hyphae varies. Some, when about half-way to the root, insinuate their apices beneath the overlapping cuticular scales of the hair, and in this manner penetrate to the interior (Pl. VI, fig. 31; Pl. VII, fig. 37). Others of the giant hyphae become closely septate at the tip and break up into groups of large spores. These groups of large spores then undergo further irregular division to form large numbers of small spores arranged in an irregular manner (Pl. VII, fig. 35). The coalescence of many groups of spores formed in this manner results in the development of the characteristic spore sheath. The notable feature of this sheath is that the spores show no trace of linear arrangement, and in surface view appear as a close mosaic (Pl. IV, fig. 13; Pl. VII, fig. 36).



Having entered the hair the penetrating hyphae proceed to grow downwards, branching continuously (Pl. VI, fig. 31; Pl. VII, figs. 34, 37). It is now conclusively shown that from the point of entrance within the intra-follicular portion of the hair shaft, the growth of the fungus is always downwards; and that, when hyphae are observed in the aerial part of the hair, their presence there is due to the upward growth of the hair carrying the fungus with it (Sabouraud, 1910, p. 198). The downward direction of growth is clearly indicated by the direction of the branches of the hyphae, which all proceed in a more or less sinuous fashion towards the root. The nearer towards the root, the more numerous and the finer these branching hyphae become, until finally they stop just above the junction of the bulb, where they form a "fringe" of fine hyphae, first described by Adamson (July, 1895) and known as "Adamson's fringe." It is quite exceptional for the bulb itself to be invaded. It was in the question of the direction of growth that Gruby fell into a serious error, as he considered that infection took place from the root end of the hair, and compared the general arrangement of the fungus to a tree, bearing branches, twigs and seeds. In addition to the intra-piliary mycelium and the spore sheath, a number of fine, branching hyphae ramify between the hair and the follicle, and never penetrate the hair or partake in the development of the spore sheath; and, beneath the spore sheath and external to the hair, there is a network of hyphae which has been called the "cuticular sheath," and is probably of importance in the formation of the spore sheath.

As the hair grows the spore sheath is renewed below as fast as it is carried up beyond the ostium of the follicle. This renewal is ensured by certain of the intra-piliary hyphae coming to the surface of the hair, projecting between the cuticular scales, and giving rise externally to groups of spores in a similar manner to that by which the giant hyphae initiate the formation of the sheath in the first instance.

The connection between the spores thus formed and the intra-piliary mycelium was long a contentious point and has not yet been satisfactorily settled. Gruby thought the terminal hyphae became covered with (lateral) spores, and this idea was later adopted by Sabouraud in place of his first theory that they were formed by abstriction one after another from the terminal hyphae. This led Sabouraud to believe that *Microsporum* produced lateral spores in the parasitic stage as well as in culture, and to consider them as therefore differing widely from *Trichophyton*; an error into which neither Adamson nor Fox fell. Finally, following Bodin, Sabouraud adopted the theory that the spores are formed by segmentation of the terminal hyphae, and this theory is now generally accepted.

According to Fox (1897) the invasion of a hair by a *Microsporum* is rapidly followed by the destruction of the cuticle which is stripped off. The development of the fungus in the hair renders it exceedingly brittle, and the infected hairs soon become broken off a short distance beyond the follicle; and the ringworm patches are usually studded with short stumps of infected hairs a few millimetres in length.

*Trichophyton*. The distinguishing characters of *Trichophyton* are that the intrapilar mycelium is composed of hyphae which are closely segmented, the segments being more or less iso-diametric, and round, oval or square in section; so that the

hyphae appear as chains of spores (Pl. IV, figs. 14, 15); and that when a circum-pilar sheath is developed the spores are always formed in chains. This chain-formation is always visible to a greater or less degree, in contrast to *Microsporium*, in which the spores of the root sheath never show a chain-like arrangement.

The primary lesion in the case of *Trichophyton* is also epidermic, and the ramifying hyphae in time reach hair follicles into which they grow. The invasion of the hair, according to Sabouraud (1910), occurs in the manner described for *Microsporium*, but Fox and Blaxall (July, 1896) observed cases in which invasion took place from the root end of the hair. Once the hair is penetrated the hyphae proceed to grow downwards towards the root, and to branch repeatedly, finally terminating just above the bulb in a fringe of fine hyphae. So far the behaviour is almost the same as that of *Microsporium*, but further development may follow one of three types, according to the species of *Trichophyton*.

In those species of *Trichophyton* called by Sabouraud "endothrix," once the invasion of the hair is accomplished the extra-pilar mycelium disappears and further development is confined entirely to inside the hair. The invading hyphae branch repeatedly, and the hair becomes filled with hyphae of uniform diameter, lying close together and parallel to each other, and growing down towards the root. These hyphae, when mature, become closely segmented and develop into chains of spores, with the result that the hair appears to be filled with regularly arranged rows of more or less uniformly shaped spores (Pl. IV, fig. 14; Pl. VI, figs. 29, 30). The important points are, that once the invasion of the hair has been effected no mycelium is seen outside the hair, and that the cuticle of the hair is not destroyed and remains for a long time enveloping the mass of spores in a bag-like manner.

In other species, called by Sabouraud (1910) "néo- or ecto-endothrix" *Trichophyton*s, in most hairs the behaviour is similar to that of the endothrix types, but a certain amount of extra-pilar mycelium is developed around some of the infected hairs. In fact, they may be regarded as endothrix forms in which the primary extra-pilar mycelium is retained for a long time.

These néo-endothrix species form a transition to the next group, termed "ectothrix" by Sabouraud (1910). Ectothrix *Trichophyton*s have caused an infinite amount of confusion in the literature, owing to the significance of the term "ectothrix," as applied by Sabouraud, being misunderstood by many authors. These authors considered that he used the term to denote fungi which lived exclusively outside the hair, while, in reality, he applied the term to all the species of *Trichophyton* which, in addition to invading the hair, always retain to a greater or less extent an extra-pilar mycelium (Pl. IV, fig. 15; Pl. VII, figs. 32). Different species of ectothrix *Trichophyton*s vary greatly in the relative degree to which the hair is invaded, and the extra-pilar sheath developed. In some cases the hair is nearly completely filled with chains of large spores, and there is a circum-pilar sheath of hyphae and spores arranged in long chains (Pl. IV, fig. 15); while in other species the extra-pilar sheath is the main feature and only the superficial layers of the hairs are invaded. These two types correspond to two groups into which the ectothrix *Trichophyton*s may be divided according to the size of the

spores. The first, or large-spored, group have spores 5-8 microns in diameter, and the second, or small-spored, group have spores 3-4 microns in diameter. In contrast to the endothrix forms, the ectothrix *Trichophyton*s soon erode the cuticle of infected hairs, although, according to Fox and Blaxall (August, 1896), the hairs are not destroyed as rapidly as by *Microsporum*s.

*Achorion*. The fungi called *Achorion* are those which cause the disease known as favus and, indeed, their definition is purely clinical, as they are defined as fungi which form the structures known as "favus cups" on the infected skin. These characteristic structures have aptly been likened to lupin seeds, and are cupuliform crusts of a yellow colour. Their structure and development were first described by Gruby, and admirably completed and supplemented by Sabouraud (1910, p. 494). Each of the so-called "cups" is formed around a hair, and is of intra-dermal origin. They originate by the accumulation of fungal elements at the mouth of the follicle in the cornified layer of the epidermis, which become compacted to form a dense ring surrounding the hair. At first it is a more or less cone-shaped mass, covered by the corneous layer of the epidermis; but by the continued addition of new elements below, aided by the growth of the hair which it surrounds, it is gradually raised above the epidermis, where the central part gradually dries and scales or powders away, resulting in the characteristic cup-like form. The peripheral region of the intra-follicular part of the cup is infiltrated with leucocytes, but otherwise it is composed of a compact mass of hyphae, of which a hair forms the axis.

The invasion of the hair is effected in the manner described for the other Dermatophytes, and the general behaviour of the intra-pilar mycelium is more or less the same, except that the hyphae are finer, more sinuous, and less numerous than in *Trichophyton* or *Microsporum*. According to the degree to which the intra-pilar mycelium forms chains of spores, the infected hair may either closely resemble one infected with a small-spored *Trichophyton*, from which it is readily distinguished by the absence of extra-pilar chains of spores, or, when the intra-pilar chains of spores are numerous, parts of the hair may resemble one infected with an endothrix *Trichophyton*, but in such cases only one part of the hair presents such an appearance, and the remainder shows the typical *Achorion* type of infection.

Infected hairs have a grey and lustreless appearance and are not rendered more fragile than normal hairs; but are peculiar in being very liable to split longitudinally, a fact which is readily observed when they are compressed between two slides. Another peculiarity of the infected hairs is that when mounted in KOH for microscopical examination, they appear to be covered with numerous small air bubbles and in the upper region of the invaded portion chains of elongated bubbles are seen inside the hair, indicating the position of hyphae which have become disorganised. Sabouraud is of the opinion that the external bubbles arise from the gradual expulsion of the internal bubbles as the KOH enters the hair.

In addition to these fungi, which are essentially parasites of the hair or hairy skin, and constitute the "ringworm fungi" in the narrow sense of the term, there

are a number of other fungi which cause dermatomycoses, but do not attack the hair. The principal of these fungi are contained in the genera *Epidermophyton* and *Endodermophyton*.

*Epidermophyton* Lang, 1879. The fungi of this genus never attack hairs but cause diseases of the glabrous skin, especially the disease known as "eczema marginatum of Hebra." They also differ from Trichophytons in that cultures never develop spiral hyphae, pectinate organs or aleuries; the only spores formed being pluriseptate "spindles" (fuseaux) of a special type.

*Endodermophyton* Castellani, 1909. This genus was founded by Castellani (1919) for another group of fungi which do not attack hairs, and are characterised by growing between the superficial and deep strata of the epidermis. They differ from most Dermatophytes in being difficult to cultivate, but cultures of several species have been obtained by Castellani (1919) by adopting a special technique. In cultures they develop a sterile mycelium and never form aleuries or spindles. In the lesions, according to Brumpt (1927), they appear as a network of hyphae, greenish yellow in colour. The hyphae are formed of cubical, rectangular or ovoid articles of irregular size; or of chains of ovoid mycelial (arthrospores) spores.

## II. CULTURAL LIFE.

Nearly all the Dermatophytes grow very readily on all the common artificial culture media; and particularly rapid and luxuriant growth is obtained on the standard media introduced by Sabouraud, which are described in the section on technique. On the glucose and maltose media growth is very rapid, and on these media the various species assume very characteristic cultural aspects by means of which, in general, the species are differentiated. On the peptone medium, on which the development of pleomorphic changes is to a great extent inhibited, growth is much slower in most species, and is not so characteristic as regards cultural form; except in the case of the small-spored ectothrix Trichophytons, the different species of which grow better on this medium than on the sugar media, and assume their most differentiated forms of cultures. The Dermatophytes grow well, but relatively slowly, at room temperature, and the optimum temperature is about 30° C. For diagnostic purposes it is customary to grow the cultures at room temperature and accept the aspect of such cultures as the normal.

On the criterion of the behaviour of the parasite with respect to the infected hair, we have seen that the Dermatophytes are divided into the genera *Microsporum*, *Trichophyton* and *Achorion*. When Sabouraud demonstrated the plurality of these genera he used the cultural characteristics as the basis of his sub-divisions and species. Thus, according to the type of cultures developed, the Microsporums are divided into two groups; the first with relatively slowly growing small cultures; and the second with rapidly growing luxuriant cultures. To the first group belong species which are of purely human origin, and to the second the species which are of animal origin. Each species, in addition to the group character of the culture, assumes, on the standard media, a cultural form which is constant and is regarded as specific for taxonomic purposes. As an example may be mentioned the commonest

*Microsporum*, *M. audouini*, which grows as a uniform finely downy felt, white towards the periphery and slightly greyish towards the centre, and with the surface marked by a number, about 4-6, of straight shallow furrows radiating from the centre and dividing the culture into sectors with almost geometrical precision (Pl. I, fig. 2).

Among the Trichophyton things are much more complicated, as each of the sub-groups, endo- and ectothrix, is further sub-divided into two or more sub-groups by the appearance of the cultures. The two principal cultural forms in the endothrix group are the "crateriform" typified by *T. crateriforme* Sab. (Pl. I, fig. 4; Pl. II, fig. 7A), and the "acuminatum" typified by *T. acuminatum* Sab. (Pl. II, fig. 5); but there are a few forms which have small glabrous cultures, and an example of these is *T. violaceum*, in which the small glabrous culture is furrowed radially and is coloured an intense violet when recently isolated, but the pigmentation gradually decreases after a number of sub-cultures. The neo-endothrix forms have cultures generally resembling the crateriform type, but instead of a "crater" the culture is thrown into an elevated mass of convolutions. The small-spored ectothrix forms are divided into the "gypseum" group of species with granulated, chalky cultures (Pl. III, figs. 9B, 10A, 11A); and the "niveum" group in which the cultures are white and softly downy (Pl. III, fig. 10B). Similarly the large-spored ectothrix forms are divided into those with large downy cultures, and those with small glabrous or "faviform" cultures (Pl. II, fig. 8B). The typical favus type of culture is developed by only one species of *Achorion*, *A. schönleinii*, which forms an elevated, yellowish, cheesy mass with a convoluted surface (Pl. II, fig. 8A). The other species of *Achorion* have cultures resembling one or other of the forms of cultures met with in the other Dermatophytes (Pl. II, fig. 7B).

### III. MORPHOLOGY.

*Mycelium.* The mycelium of the Dermatophytes is composed of branched septate hyphae, having an average diameter of 4-5 microns, and not exhibiting any marked peculiarities or differing essentially from the mycelium of common saprophytic fungi. Ota and Langeron (1923) give a very concise and lucid account of the various forms of spores and other mycelial structures developed, and it is on this paper that the arrangement of the following description of the most important organs, reproductive or of doubtful significance, is based. As will be seen later, the general classification into *Microsporum*, *Trichophyton* and *Achorion* has no real botanical basis, and, from a morphological point of view, these genera are of no significance as regards the relationship of the different species. For this reason the various kinds of spores and other organs will be described in a general manner without regard to their occurrence in any particular genus or group. The distribution of the different spores and other organs will be discussed in the section on classification.

First, a few more or less peculiar structures developed by some species may be described. Of these the principal is what Sabouraud called "racket-shaped hyphae," which consist of hyphae each cell of which becomes greatly enlarged at one end,



probably always the distal end, with the result that they suggest the appearance of a chain of tennis rackets placed end to end (Pl. VI, fig. 22). Various other structures described by Sabouraud are: "terminal clubs," consisting of hyphae the apices of which become swollen; and "chandeliers faviques," which are similar structures developed in bunches owing to a number of short terminal branches all swelling at the same time.

These structures have been interpreted recently by Grigoraki (1925) as being the final stage of degeneration of reproductive organs resulting from pleomorphic changes induced by cultivation on artificial media. Here may also be mentioned some other organs of doubtful significance. Pectinate, or denticulate, hyphae are hyphae, usually curved, bearing on one side a number of short projections which give a pectinate or denticulate appearance according to the length to which they are developed (Pl. VI, figs. 23-26). Matruchot and Dassonville (1899) attached taxonomic importance to these structures and regarded them as representing hyphal ornamentations such as are found on the peridia of certain Gymnoascaceae; but Grigoraki regards them as arising from the development of a number of aleuries or chlamydospores along one side of a hypha, which then becomes shrunken and contracted between the points of insertion of the spores, the pectinations finally marking where the spores had been formed. "Nodular organs" are found in cultures of some species, and generally appear as pectinate hyphae the pectinations of which develop into short hyphae; or, more rarely, they appear as hyphae from the terminal region of which masses of short hyphae grow intertwining in all directions; and in still other cases they consist simply of masses of interwoven, sinuous hyphae (Pl. VI, fig. 27). Some authors consider that these nodular organs represent vestigial or abortive peridia. Finally, in some species "spiral hyphae," consisting of hyphae the terminal part of which is coiled into a close spiral, are formed (Pl. VI, fig. 28), and these are also regarded as being of taxonomic importance and are considered to represent peridial ornaments such as are found in some Gymnoascaceae.

*Spores.* Arthrospores are a very common form of reproduction in the Dermaphytes, and may be considered to be the sole means of reproduction in the parasitic stage in the hairs. In culture they are generally formed in chains by the segmentation of terminal hyphae, and the rounding off and separation of the individual cells, forming what Sabouraud called "false spores in chains" (Pl. V, figs. 1, 2). In some cases each more or less rounded cell becomes sub-divided into several other cells each of which becomes an arthrospore. This type of spore is developed when conditions for growth are adverse owing to the drying up of the medium or some other cause. Under certain conditions arthrospores are developed by higher fungi such as *Aspergillus* and cannot, therefore, be used in classification.

Chlamydospores of many types are formed. The first type, called an "endoconidium," may be regarded as a small chlamydospore, and consists of a vegetative cell which has become thick-walled and has increased slightly in size at the expense of its neighbours (Pl. VI, fig. 18). True chlamydospores attain very considerable sizes, and are of two main types, intercalary and lateral (Pl. VI, figs. 20,

21). Lateral chlamydospores are usually shortly pedicellate, and constitute what Sabouraud described as "pedicellate chlamydospores" (Pl. VI, figs. 17, 19). The spore may or may not be separated from the pedicel by a septum; and, similarly, the pedicel may or may not have a septum cutting it off from the hypha, and both spore and pedicel may be continuous with the hypha. Vuillemin (1910) applied the term "aleurie" or "aleuriospore" to the somewhat more differentiated spores formed by most Dermatophytes. These are external spores, but differ from true conidia in that they develop at the expense of the hypha from which they arise, the protoplasm of the latter migrating into them as they develop; and in the fact that they are not abstricted and become free only by the death and disintegration of the mother hypha, vestiges of which usually remain attached to the free aleurie at the point where it had been connected with the hypha. Many very different forms of aleurie are found in different species. Sometimes they are continuous with the hypha, and sometimes they are separated from it by septa; and in the latter case they may be sessile or attached by a small denticulation. They may be lateral, terminal, or even intercalary (Pl. V, figs. 3-6). When they are lateral, several may be developed in a chain and form a transition to the type of spore known as "spindle-shaped chlamydospores" or "fuseaux," to be described presently. The aleuries may be borne on simple hyphae or on hyphae branched to varying degrees. Sometimes the branches are so numerous, and the aleuries so abundant, that they form dense bunches suggestive of bunches of grapes, and it is to these that Sabouraud applied the term "grappes conidiennes."

Spindle-shaped chlamydospores, which are also called terminal chlamydospores, and, by Sabouraud, "fuseaux" or "pluri-septate fuseaux," are the most important reproductive structure formed by the Dermatophytes. They are formed in great abundance in certain groups, rarely in others, and not at all in some. They will be referred to here as "spindles" for the sake of brevity. These spores are large, terminal, thick-walled spores, which are generally more or less spindle-shaped and are divided by septa into a number of loculi. They are very variable in size and form and may be aseptate (Pl. V, figs. 9, 16); or have 1, 2 or many septa (Pl. V, figs. 7, 8, 10, 13-15); they may be comparatively thin-walled or very thick-walled (Pl. V, figs. 14, 8); and may be smooth, or covered, completely, or in part, with spines or short blunt protuberances. Sometimes they terminate in a long whip-like prolongation. In the various forms they show a transition from, in some species, chains of chlamydospores; and in other species from lateral septate aleuries. Ota and Langeron (1923) consider that they have evolved from spindles or aleuries in this manner; but Grigoraki (1925) holds the opposite view and considers that the spindle is the normal form of spore, and that aleuries and chlamydospores are the final stages in the pleomorphic degeneration of the spindles. This view will be discussed in detail in the section on classification, and in the sub-section on pleomorphism. These spindles resemble the spores found in *Blastotrichum*, and according to Lindau (cited by Ota and Langeron, 1923) they are characteristic of the family Hyalophragmideae of the Mucedineae; and, together with the aleuries, are very important from the taxonomic point of view.

## IV. SAPROPHYTIC LIFE.

The ease with which the Dermatophytes may be grown on artificial culture media, and the ready manner in which, under certain conditions, they develop spores, is considered by many authors, and especially by Sabouraud (1910, p. 726), as evidence that they occur as saprophytes in nature, and that the parasitic stage is only a transient and more or less accidental phase in their life history. This view is supported by the fact that many vigorous forms, especially the small-spored ectothrix Trichophytons, may be readily grown on substances such as sawdust, rotten wood, and grain. Indirect proof was afforded by Matruchot and Dassonville (1901), who succeeded in producing a form of ringworm in a dog by inoculation with a common saprophyte, *Ctenomyces serratus*, one of the Gymnoascaceae, to which family they consider the Dermatophytes belong.

Recently very interesting work on these lines has been done by Nannizzi (1926). He first demonstrated that some Dermatophytes, *Trichophyton granulosum*, *T. denticulatum*, *T. felineum*, *T. equinum*, *T. radiolatum*; *T. asteroides*, and *Microsporum lanosum*, can grow as ordinary saprophytes on feathers, pelts of guinea-pigs, human hair and leather, if kept moist and dark, at ordinary temperature. He also grew *T. radiolatum* on a dead guinea-pig interred in a wooden box; and *T. asteroides* on forest soil mixed with feathers and bones and freely exposed to light. Having demonstrated the possibility of saprophytic growth in this manner, he proceeded to investigate such growth under more critical conditions, by growing some of the species—*T. asteroides*, *T. radiolatum*, *T. denticulatum*, and *T. felineum*—in tubes on sterilised leather, feathers and hair. They grew readily under these conditions, and he found that on such sub-strata these species developed globular organs which he considers to be pycnidia, corresponding to the peridia of the Gymnoascaceae. These organs differ from the peridia of the Gymnoascaceae in the fact that, while the general morphology is similar, they do not contain true asci, but spores similar to the aleuries, which are termed pycnospores. Various species of the different genera of the Gymnoascaceae were studied comparatively when cultivated in a similar manner on feathers, bones, hair and so on. In all cases they were found to form bunches of aleuries, "spindles" and various other organs similar to those formed by the Dermatophytes. On very rich media he found that the peridia in these fungi did not remain globular but spread out and remained dispersed, a phenomenon which he says is very common in fungi forming peridia and sporodochia, and which he has observed in the genera *Volutella*, *Fusarium*, *Coremium*, *Coniosporium*. This phase of dispersed perithecia closely resembles what is found in the Dermatophytes on ordinary media, with the difference that the Gymnoascaceae, when reinoculated on poor media, revert to the normal form of peridia; while the Dermatophytes always have the dispersed form on artificial media whether rich or poor, and develop the rudimentary peridia only when grown saprophytically on substances such as hair, feathers and leather. Pleomorphism is also known to occur in *Ctenomyces serratus*, as well as in various Hyphomycetes, including *Stemphylium*, *Alternaria*, and *Macrosporum*, when they are grown on

artificial media rich in carbohydrates. His final conclusion is that there is sufficient evidence for regarding the Dermatophytes as Gymnoascaceae, and he considers that they are forms of Gymnoascaceae which in culture present a kind of teratological aspect, shown by the dispersed peridia and induced by the abnormal conditions of growth. In consequence of this opinion he reviews previous classifications of the Gymnoascaceae, and proposes a new arrangement, in which the Dermatophytes are included, details of which will be given in the section on classification.

More recently Nannizzi (1927) has found that when *Achorion gypseum* is grown at laboratory temperature on sub-strata of animal origin, peridia are formed which contain true asci each of which contains 8 oval, hyaline ascospores.

I have tried to verify the development of the peridial-like organs described by Nannizzi by growing a number of species on strips of the pelt of a guinea-pig, and on feathers of fowl. The species grown on the pelt, which was cut in strips and the strips placed in test-tubes containing a few c.c. of water in the end, and sterilised by autoclaving for 30 minutes at 120° C., included *T. granulosum*, *T. felineum*, and *T. radiolatum*, normal and pleomorphic forms, in all of which he observed the development of the globular organs. All the species tried grew well, both at room temperature and at 25° C., but in no case were any structures resembling those described by Nannizzi formed. The feathers were arranged in the same way in test-tubes having a few c.c. of water in the end, and were inoculated with the species: *T. radiolatum*, normal and pleomorphic forms, *T. granulosum*, *T. laticolor*, *T. farinulentum*, and *T. radians*, all of which belong to the group of small-spored ectothrix Trichophytos, to which the species found to produce peridia by Nannizzi belong. All the species again grew very well, but up to the time of writing none has developed any globular organs resembling peridia (Pl. III, fig. 12 A, B). It is not considered that these results are contradictory to Nannizzi's to any serious degree, as the cultures used were obtained from Dr Sabouraud over two years ago, and it is probable that the tendency to develop "peridia" may be gradually lost when the fungi are kept a long time in culture. It is also possible that there are certain strains of these species which form the "peridia" more readily than others.

Other Dermatophytes were grown on the same guinea-pig pelt, including the species *T. crateriforme* (two strains, one supplied by Dr Sabouraud, and one freshly isolated), *T. sulfureum* (freshly isolated), *M. lanosum* (freshly isolated), and *M. audouinii* (freshly isolated). All of them grew well, but none developed any organs suggestive of peridia. It was also found that when such cultures were reinoculated on the ordinary sugar media the original form was obtained, and they did not turn pleomorphic when grown under those conditions on the pelt.

## V. CYTOLOGY.

The cytology of the Dermatophytes was almost completely neglected until recently, when Grigoraki (1925) made an extensive study of the cytology of the group, and utilised the results as a basis for a new classification of the Dermatophytes. So greatly was this side of the subject neglected by previous workers,

that Grigoraki is able to give references to only three short notes which contain the results of all previous work on the cytology.

The results of Grigoraki's investigation may be summarised briefly as follows: the fungi studied may be divided into two groups: one, with a mycelium formed of segments each of which contains several nuclei; and, two, with a mycelium formed of uninucleate cells. To the first group belong *Achorion*, *Microsporum*, *Trichophyton*, *Epidermophyton*, or, in fact, all those fungi which are ordinarily regarded as "ringworm" fungi; and to the second belong *Malassezia*, *Sporotrichum*, *Madurella*, and *Aleurisma*; which are fungi also causing dermatomycoses, but which are not commonly regarded as ringworms.

In all cases the nuclei exhibit the general structure found in fungi, and consist of a hyaline nucleoplasm delimited by a more or less colourless membrane and containing a large nucleolus. Chromatin is only seen in very favourable conditions, and then appears as a reticulum or as granules. Nuclear division is apparently amitotic. The number of nuclei in multinucleate segments may be 2-6, or even 10 in the case of the apical segments of growing hyphae. The aleuries and conidia contain a single nucleus, while chlamydospores and arthrospores have 2-5 and the loculi of the "spindles" have a similar number.

In the cytoplasm there are very refringent granules, at first small and few in number, and later enlarging and uniting to form large globules. These granules darken with osmic acid and are of a lipid nature. They appear to serve as reserve substances in early stages of growth, but in later stages a kind of fatty degeneration seems to occur, and very old hyphae have the cytoplasm nearly completely filled with these globules.

The presence of mitochondria is readily revealed by using the appropriate technique; and they are sometimes visible as little refringent filaments which stain vitally with janus green or dahlia violet. They usually appear as filaments of various lengths resembling those found by Guilliermond in various other fungi.

In all the fungi vacuoles are present, the contents of which stain selectively with most vital stains, especially with neutral red. These stained corpuscles in the vacuoles also appear with stains such as haemalum, Unna's blue, cresyl blue, and methylene blue, after fixation with formol, picroformol or alcohol, but not after mitochondrial fixatives. They agree in all particulars with the metachromatic corpuscles described by Guilliermond, Meyer, etc., and thus represent the Golgi apparatus. A study of these corpuscles confirmed Dangeard's view that they are formed by the vital stain or fixative causing the precipitation of a substance normally present in the vacuoles as a colloidal solution and called metachromatin by Guilliermond.

Glycogen was found to a greater or less degree in all the fungi examined; and is especially abundant in spindles, chlamydospores, and arthrospores, but is not found in aleuries. No relation between the glycogen and mitochondria was observed.



## VI. PLEOMORPHISM.

Pleomorphism is the name given by Sabouraud to a phenomenon which is very prevalent among the Dermatophytes and is almost peculiar to them. It is a kind of cultural change or degeneration, and has a superficial resemblance to what is known as "sectoring" or "mutation" in certain other fungi such as *Fusarium* and *Phoma* (Brown, 1925; Chodat, 1926). The fungi in which it occurs, when inoculated on suitable medium, grow and develop normally until a certain stage is reached, usually in about four to six weeks, when the colony has reached its maximum development, and then white downy tufts of mycelium suddenly appear growing on the surface of the mature colony. These tufts may arise at any point, but most commonly they first appear in the central region of the colony. They rapidly grow and spread and finally unite together to form a carpet of pure white downy mycelium covering the whole surface of the primary culture. This downy, or pleomorphic, mycelium may be inoculated on to fresh medium, and cultures obtained of the pure pleomorphic mycelium, without any of the primary or normal type of culture (Pl. III, fig. 11 B, cf. fig. 11 A). In some cases, where the pleomorphic form is not fully developed, mixed cultures, partly normal and partly pleomorphic, may be obtained.

Microscopically the pleomorphic mycelium differs markedly from the normal, and is composed of long, fine hyphae, the protoplasm of which is much more vacuolated, and contains more numerous fat globules than the hyphae of the normal mycelium (Grigoraki, 1925). The most striking fact, however, is that the pleomorphic mycelium is completely sterile in the majority of cases. Sometimes the degeneration is not complete, and elongated hyphae bearing small, little-differentiated, sessile, lateral spores are formed, and sometimes chlamydospores are developed. With the exception of these simple lateral spores and chlamydospores, no reproductive organs such as are found in the normal mycelium, aleurias, spindles, pectinate and spiral hyphae, etc., are developed by the pleomorphic mycelium.

Different Dermatophytes vary widely in the tendency to undergo pleomorphic changes. Pleomorphism is especially common in the small-spored animal Trichophyton of Sabouraud, and in the quickly growing animal Microsporums; and is rare in the human, or "crateriform" Trichophyton. In some species, notably *Microsporum audouini*, the commonest of the ringworm fungi in England, it is not known to occur. Pleomorphic forms of the downy cultured animal Trichophyton (*T. radians* and *T. denticulatum*) are not known, and, as these species grow as white downy colonies, and have very simple reproductive organs, Sabouraud has suggested that they are naturally occurring pleomorphic forms of some of the animal Trichophyton.

In culture the pleomorphic forms grow even more readily than the normal, and may be kept in culture indefinitely; but a pleomorphic form has never been known to revert to the normal form once it has been separated as a pure culture. The pleomorphic forms of most of the different species are very similar to each other, and, with the exception of a few cases where the pleomorphic forms have charac-

teristic cultural aspects, it is not possible to tell the pleomorphic form of one species from that of another. This fact caused endless confusion in early work on the Dermatophytes before the phenomenon was recognised.

According to Sabouraud, pleomorphism is greatly influenced by the conditions of growth, and, in general, is most prone to occur when conditions for growth are most favourable. It is apparently very dependent on the presence of carbohydrates—sugars or glycerin—in the medium, and occurs most readily on media containing a high concentration of carbohydrates: about 4 per cent. On the other hand, nitrogenous substances tend to prevent pleomorphic changes, and the higher the ratio of nitrogenous food (peptone) to carbohydrates, the less readily do cultures turn pleomorphic. Sabouraud has taken advantage of this fact to introduce what he calls a "preserving medium," on which pleomorphism does not usually occur, by excluding all carbohydrates and making up the medium with 3 per cent. of peptone. Other factors which tend to stimulate the rapidity of growth also favour pleomorphism, such as growing cultures at temperatures of from 30°–37° C.

In nearly all cases pleomorphism is manifested by a pure white downy culture; but in some forms there are several different kinds of pleomorphic cultures. In *Microsporum lanosum* this is most marked, and there are three quite distinct pleomorphic forms. Pleomorphism may appear as a white downy carpet very similar to pleomorphic forms of other animal Microsporums; or it may be an immersed brownish glabrous culture; or it may be a coarse, shaggy, downy culture. The last form is the commonest and the most stable, but the three forms are all reversible to each other, but not to the primary or normal form.

According to Sabouraud the pleomorphic form when inoculated on animals behaves in exactly the same manner as the normal form, and is indistinguishable from it throughout the parasitic phase. In spite of this, if the fungus is recultivated from the infected hairs or scales, it invariably grows as the pleomorphic form; and, moreover, the pleomorphic form thus obtained is at exactly the same stage of pleomorphism as was the culture with which the animal was inoculated. If the culture was completely pleomorphic and quite sterile, the cultures obtained from the hairs or scales are likewise; but if the original culture developed the rudimentary lateral spores which are found in incompletely degenerate forms, such spores are developed when it is recultivated after a period of parasitism, during which it in no way differs from the normal form of the fungus.

Sabouraud at first thought the phenomenon was a case of commensalism, or growth of two different fungi of which one was the true parasite. This view was adopted by many workers and was maintained until 1895, when Bodin, as a result of his work on a *Microsporum* of the horse, denied that it was a case of commensalism. Then he and Sabouraud together elucidated the matter, and Sabouraud verified it for the different species of *Trichophyton*, and made public his results at the Congress of London in 1896. In the same year Fox and Blaxall (Sept. 1896) expressed their disbelief in commensalism and agreed with Bodin that the white downy tufts represent "sports" or possibly degenerate forms of the fungus itself, of which it was a "pleo-morphism."

Grigoraki (1925), as a result of his cytological work on the Dermatophytes, came to the conclusion that pleomorphism is a progressive degeneration which all the Dermatophytes undergo owing to the adverse conditions of saprophytic life on artificial media. He considers it to be a phenomenon "special" to the Dermatophytes, although it is found to a very much less degree in some other fungi. He also considers that it occurs on all media, even on medium rich in peptone and devoid of sugars, but on such media it is very much slower than on media rich in sugar. This is confirmed by my own experience, for several species have turned pleomorphic although kept on Sabouraud's peptone medium, and include the species *T. asteroides* and *M. gypseum*.

Grigoraki's "progressive degeneration" theory may be regarded in two ways: (1) pleomorphic changes occurring with the ageing of a single culture; and (2) pleomorphic changes gradually developing over a number of sub-cultures. The degeneration consists in the gradual simplification and reduction in number of the reproductive organs. First the spindles become less numerous and less differentiated, while aleuries and chlamydospores become more numerous. Then the aleuries and chlamydospores in turn gradually degenerate, until the final phase of pleomorphism is reached, which consists of a downy mycelium of fine, vacuolated, sterile hyphae. This course of events may take place in a single culture. First spindles are numerous, then more and more aleuries and chlamydospores are formed while the spindles decrease in number, and, finally, the sterile white downy mycelium arises. When the fungus is regularly sub-cultured the process is delayed as each new culture starts under more favourable conditions; but there is a gradual and progressive degeneration from culture to culture.

Grigoraki (1927) has recently brought forward evidence as to this degeneration of the spindles in culture, by showing that when certain Trichophyton (small-spored ectothrix of Sabouraud), which he classed in the genus *Spiralia*, are freshly isolated from infected hairs or scales, the primary cultures so obtained develop very numerous spindles during the first five days in culture. Then the spindles give rise to hyphae bearing aleuries and chlamydospores, which become very numerous, and the spindles are produced in decreasing numbers. He bases a new classification on this conception of pleomorphism, in which he considers that only the primary cultures obtained directly from infected hairs or scales are of value in determining the taxonomic characters of the Dermatophytes. This question is discussed in more detail in the section on classification.

Nannizzi (1926) has observed pleomorphism to occur in *Gtenomyces*, a fact which he regards as evidence in favour of the relationship of the Dermatophytes and Gymnoascaceae, and also in certain Hyphomycetes, such as *Stemphylium*, *Alternaria*, and *Macrosporium*, when they are grown on artificial media rich in carbohydrates.

The essential point in pleomorphism is that the same species invariably gives rise to the same pleomorphic form; and, in the species which are prone to pleomorphism, it develops constantly and regularly under suitable conditions. It is in this that the phenomenon differs most strikingly from what is known as sectoring or

mutation in other fungi. Chodat (1926) made an extensive investigation of sectoring and mutation in *Aspergillus ochraceus* and in *Phoma alternaria-acearum*, and his results are convenient for comparing this phenomenon with pleomorphism. The mutation studied in *A. ochraceus* was reversible to the normal form and is not comparable to pleomorphism. In *Phoma*, however, the mutations were irreversible, and, although both the parent form and the mutations gave rise to other mutations which approached each other more closely than the parent forms, in no case was there a reversion to the primary or normal form. The mutations arose sporadically as sectors, during the development of the colonies; and under normal cultural conditions the percentage of cultures developing sectors was only 1-2 per cent. There is no record of the same mutant occurring repeatedly, and this fact is in striking contrast to what occurs in pleomorphism. The differences of the sectors from the normal form, or parent culture, were comparatively small, and consisted in slight physiological differences in the ability to utilise carbohydrates, in the production of glycogen and a dark pigment, and in the development of a mycelium rich in "hypnocysts" or muriform spores. The sectors occurred most abundantly on poor media, and Chodat considers that the medium does not exert any causative action as regards their development, but merely allows latent mutations to become apparent.

It is evident from these facts that almost the only thing in common between sectoring and pleomorphism is that each is an irreversible deviation from the normal form of mycelium. Sectors arise during the growth of the colonies and are strictly localised, while pleomorphic forms, regarding the phenomenon in the narrow sense as the appearance of the white downy mycelium, develop as an overgrowth on the culture, and may arise all over its surface. Pleomorphism entails the degeneration of mycelium and reproductive organs; and, almost invariably, there is a complete lack of pigmentation in pleomorphic forms of pigmented species; while the sectors of *Phoma* in some cases showed increased pigmentation and spore production. Finally, the mutant sectors may themselves give rise to other mutations, in contrast to the pleomorphic forms of the Dermatophytes, which are exceedingly constant, except in the few rare cases where there are several interchangeable pleomorphic forms.

### 3. PHYSIOLOGY.

Hitherto the majority of workers have completely neglected the physiology of the Dermatophytes, and most of the work that has been done on the subject dates from the time before Sabouraud had established the differences between the various forms of ringworm; and, beyond that the fungi dealt with were isolated from cases of ringworm, in most cases it is not possible to tell what species were used or even whether they were Trichophytions or Microsporums. Sometimes the fungi were described as having *Aspergillus* fructifications (Roberts, 1894) and were obviously ordinary saprophytes. Even in the few cases where the work is reliable only one or two forms were dealt with and the scope of the enquiry was very limited.

The first paper dealing with the physiology is by Verujsky (1887), who made a

comparative study of the morphology and physiology of "*Trichophyton*" *tonsurans* and *Achorion schönleinii*. He found that a neutral or a slightly acid medium is most favourable for growth, the optimum temperature being about 33° C. Spores of *Trichophyton tonsurans* are killed by heating in distilled water to 49° for 10 minutes, but *Achorion* is slightly more resistant and spores may germinate after 10 minutes at 50°. He also found that minute traces of all the ordinary antiseptics kill the cultures and spores. Both liquefy gelatin, *Achorion* more rapidly. *Achorion* does not utilise sugar in any medium, but assimilates large amounts of nitrogenous substance (urea); while *Trichophyton* utilises glucose with the formation of oxalic acid as an intermediate product, but does not invert saccharose. On malt medium the ratio for *Trichophyton* of weight of fungus : to sugar consumed is 1 : 2. The presence of glycerin in the medium increases the ratio to 2 : 3.

Macfadyen (1894) demonstrated the presence in *T. tonsurans* of a proteolytic enzyme actively liquefying gelatin, which can act apart from the living mycelium. It acts in both acid and alkaline medium, but is most active in the latter. Neither diastase nor invertase could be demonstrated, and the fungus neither grew in milk nor did the cultures containing the enzyme curdle it. Fibrin was not acted on by the enzyme. The fungus grew on pure keratin obtained from quills, and thereby rendered it more soluble in strong alkali, but a definite enzyme acting on keratin was not demonstrated.

Roberts (1894) found that various kinds of hairs, animal and human, were attacked to a varying degree when placed in growing cultures of *Trichophyton* and *Achorion*; some destroyed the cuticle and cortex simultaneously, and some first attacked the cortex, and only later, if at all, the cuticle. He proposed that this "keratolytic" power should be used in classifying the Trichophytons. Later (1899) he found a proteolytic enzyme in *Trichophyton* cultures 6 years old.

Bodin (1899) found that, for what he described as the oospora form of the *Microsporum* of the horse (*M. equinum* Bodin, in Sabouraud), the optimum temperature for growth is about 35° C., and that glucose, dextrin and maltose are assimilated in order of choice; but sucrose is not utilised. Later (1901) he found that the same fungus produces an enzyme in the culture fluid which clots milk, and another which dissolves the clot. The latter, casease, is most abundant in neutral peptone-glucose medium at the moment when the glucose has been completely consumed. The fluid containing casease also liquefies gelatin, and, to a much less degree, hydrolyses egg-albumen and coagulated serum. In 1902 he found that *Achorion quinckeanum* also utilises glucose more readily than lactose or maltose; and that, after growth, the culture fluid contains casease, rennin (clotting milk) and gelatinase. Later (1907, cited in Sabouraud) he described four proteolytic enzymes in *Achorion gypseum*: (i) trypsin, acting on coagulated albumen and most abundant in the pleomorphic form; (ii) gelatinase, also more abundant in the pleomorphic form; (iii) rennin, which coagulates milk; and (iv) casease, which dissolves the coagulum and is more abundant in the normal form.

Sabouraud cites a number of observations on the resistance of the fungi in the scales and hairs. From these observations it appears that the fungi are always dead



after 2 years; but positive inoculations of animals were obtained with material 18 months old. Railliet found that material 3 months old no longer produced ring-worm by simple rubbing; but gave positive results after 6 months when inoculated by scarification. Material which has ceased to give positive results with animal inoculation may easily give cultures. Sabouraud found the *Microïdes* to be much more resistant than most other Dermatophytes. Sabrazès (cited in Sabouraud) found spores of *Achorion* in favus cups still living after 2 years. While no antiseptic kills the fungus while parasitic in the living hairs, in cultures they are very susceptible to traces of any of the usual antiseptics, chloroform, formalin, corrosive sublimate, iodine, carbolic, etc. In the scales they are also easily destroyed, but are more resistant in the extracted hairs. The vitality of cultures under ordinary conditions is very great. I have made sub-cultures from tube cultures on Sabouraud's peptone-agar over 1 year old. Sabouraud finds that endothrix Trichophytons live about 6 months, and that the small-spored ectothrix Trichophytons may be alive after 2 years. He also finds that the pleomorphic forms differ from the normal in this respect and rarely live longer than 4-6 months without sub-culturing.

When grown on carbohydrate-containing media, many Dermatophytes produce pigments. The most remarkable is that of *Sabouraudites gallinae* when grown at 30° C. on glucose or maltose medium; a bright red pigment is formed which diffuses out into the medium. Red pigments are also found in *T. megnini*, *T. vinosum*, *Bodinia violacea*, and *S. violaceus*. The crateriform and gypseum Trichophytons often develop yellowish or reddish brown pigments, especially when the cultures are old and drying up; and many Microsporums form yellow or brown pigments—*S. lanosus*, *S. felineus*, *Grubyella ferruginea*. Sabouraud, apparently quoting from Truffi, says these pigments are acids which are precipitated by alkalis; and are different from the pigments of bacteria which are lipochromes.

Recently I investigated the comparative physiology of a number of species representing the main groups in Sabouraud's system; and also the physiology of the normal and pleomorphic forms of one species. The results of this work will shortly be published in detail<sup>1</sup>, but a brief account of the general results may be given here.

The Dermatophytes which were studied are: *Sabouraudites radiolatus* (= *Trichophyton radiolatum* Sabouraud); normal and pleomorphic forms: *S. lanosus* (= *Microsporum lanosum* Sab.); *S. audouini* (= *M. audouini* Gruby); *Trichophyton tonsurans* (= *T. crateriforme* Sab.); and *Grubyella schönleinii* (= *Achorion schönleinii* Remak). *Aspergillus niger*, *A. fumigatus*, *A. ochraceus*, and Baker's yeast (*Saccharomyces cerevisiae*) were used as representatives of common saprophytic fungi for comparison with the Dermatophytes. Most of the work was devoted to studying the enzymes present in the different species, chiefly by means of powders resembling Buchner's "zymin" preparation from yeast (Onslow, 1923), obtained from the mycelium by treatment with acetone and ether. As a preliminary to the work on enzymes, the general physiology was to some extent investigated.

It was found that when grown on synthetic media (modified Dox's medium) the Dermatophytes can utilise equally well nitrates and ammonium salts as sources

<sup>1</sup> *Parasitology*, in the press.

of nitrogen; but they cannot utilise sodium acetate, formate or lactate as sources of carbon. On both synthetic and Sabouraud's media they have a wide pH range, the limit for growth on the acid side being about pH 3.0-4.0; while on the alkaline side the limit was not determined but is beyond pH 8.0. The optimum pH is about pH 6.0-7.0; and the maximum concentration of phosphates in buffered medium for growth is about  $M/60$ .

In all the fungi studied the oxidase system resembles that recently demonstrated in Baker's yeast by Keilin (1927), and consists of a peroxidase and an indophenol oxidase coupled with a certain reductase activity. Catalase is present in all the species; but tyrosinase was not detected in any. The oxidase system of these fungi therefore differs from the oxygenase-catechol-peroxidase system of higher plants (Onslow, 1923) and from the direct oxidase present in Basidiomycetes (Robinson, 1924; Onslow and Robinson, 1926).

All these Dermatophytes were found to possess an active proteolytic enzyme, which acts in an alkaline medium and can hydrolyse intact proteins (casein) with the production of free amino-acids (tryptophane). Thus, this enzyme resembles trypsin. Pepsin was not found in any of the Dermatophytes. This is in striking contrast to *Aspergillus niger* and Baker's yeast, in which the proteolytic enzyme acts in a strongly acid medium, and more resembles pepsin. The amount of trypsin present varies in the different species, and it is particularly abundant in *Sabouraudites radiolatus*. A keratolytic enzyme could not be demonstrated in any of the fungi tested. They all have an active lipase, which readily hydrolyses tributyrin. Urease is present in all with the notable exception of *Trichophyton tonsurans*.

Of the carbohydrases studied, invertase, lactase, zymase and inulase were not found in any of the Dermatophytes. Maltase and diastase are present in all to a varying degree, being most abundant in *Trichophyton tonsurans* and least in *Sabouraudites radiolatus*. It appears that the general carbohydrase activity varies inversely with the proteolytic activity, those species which have most trypsin have least carbohydrases, and those with least trypsin have the most active carbohydrases. Amygdalase is present in all the species.

The comparison of the enzymic activity of the normal and pleomorphic forms of *S. radiolatus* showed that there is practically no difference between the two forms as regards the respiratory enzymes. The normal form, however, has a greater proteolytic activity than the pleomorphic, while the pleomorphic form has decidedly more urease and amygdalase than the normal form.

The pigments produced by the species *Trichophyton megnini*, *T. vinosum*, *T. acuminatum*, *Sabouraudites ruber*, and *S. radiolatus*, were examined and compared with the pigment produced by *Aspergillus ochraceus*. The Dermatophyte pigments are red to reddish-brown as they occur naturally, and the *Aspergillus* pigment is yellow. They are all easily soluble in dilute acids and acid alcohol, but only very slightly soluble in dilute alkalis. The colour in acid solution is yellow and changes to red or reddish-brown in alkaline solution, the colour change being reversible. They are not destroyed by boiling, and the acid solutions pass into ether and chloroform. Alkaline solutions reduce to a clear yellow with sodium

hydrosulphite, and reoxidise to red in contact with air, and this reduction and oxidation may be repeated many times. In their general properties they resemble the yellow pigment found in lichens of the genus *Physcia*, and they appear to be of the nature of anthracene pigments<sup>1</sup>.

#### 4. CLASSIFICATION AND SYSTEMATIC POSITION OF THE DERMATOPHYTES.

*Sabouraud*. Early workers classed the fungi of ringworm as oosporas, but the first real attempt at a classification of these fungi may be said to be that of Sabouraud. Sabouraud at first considered Trichophyton and Microsporum to belong to different families, but later he conformed to the opinion of Fox and Adamson that they were both closely related, and he finally placed them together with *Achorion* in one family. He considered that they were Hyphomycetes related to forms like *Sporotrichum*, and formed part of the family Mucedineae. The classification which he elaborated has little real botanical or mycological basis, and depends on clinical characters, the manner in which hairs are invaded, and on the macroscopical appearance of the cultures on certain standard media. On the first two grounds the Dermatophytes are divided into the genera *Trichophyton*, *Microsporum*, and *Achorion*, the distinguishing characters of which have been mentioned in the section on morphology. *Trichophyton* is divided into the groups endothrix, néo-endothrix and ectothrix, according to the manner in which they attack the hair. The endothrix and néo-endothrix are small groups of species, which are differentiated by the characteristic cultural aspects developed on the standard media. The ectothrix is a large group which, according to the size of the spores formed in and around the infected hairs, is divided into the sub-groups, small-spored or microides, and large-spored or megalosporon. The small-spored forms are subdivided into the "gypseum" group with dry granular, chalky-looking cultures (Pl. III, figs. 9A, B, 10A, 11A); and the "niveum" group with cultures which have a white downy appearance (Pl. III, fig. 10B). The large-spored group is divided into those with downy cultures, and those with "faviform" cultures (Pl. II, fig. 8B), or cultures which have a glabrous waxy appearance like cultures of *Achorion schönleinii* (Pl. II, fig. 8A), the fungus which causes most cases of favus. Achorions are classed as human forms, or forms known to occur only on human beings, of which *A. schönleinii* is the only species; and animal forms which infect both man and animals and of which several species are known (Pl. II, fig. 7B). Microsporum are divided into: (1) human forms with slowly growing small or medium-sized cultures (Pl. I, fig. 2); and (2) animal forms with large rapidly growing cultures (Pl. I, figs. 1, 3).

While this classification is undoubtedly very convenient from a practical point of view, and is especially valuable in medical work, it is very far from being a really scientific classification; and, when the botanical characters of the fungi are considered, it is found that species which closely resemble each other in morphology

<sup>1</sup> In connection with the work on pigments I am deeply indebted to Mr R. Hill, Biochemical Institute, Cambridge, for his help and advice.

may be placed in widely separated groups in Sabouraud's scheme. Another great objection to it is that the characters on which it is mainly based, such as endothrix or ectothrix behaviour in the hair, or the development of favus cups, are now known to be inconstant. The mode of development of the fungus in the invaded hair is probably as much regulated by the structure of the hair as by the species of fungus, and in some cases a species may be a pure endothrix in one animal and a pure ectothrix in another. Kato (1926) has isolated a species (*T. coccineum*) in Kiushiu which is a pure endothrix in man, but behaves as an ectothrix when inoculated to guinea-pigs, rabbits and dogs. Similarly, there are forms of favus known where favus cups are developed capriciously, sometimes they may be developed in a normal manner, sometimes they may be few and atypical, and in some cases they may be completely absent. Hashimoto, Ishibashi, Iwatake and Ota (1927) have described a variety of *A. schönleini* (var. *mongolica*) which may produce typical favus cups, or small cups, or, as is most common, the cups may be completely absent and the clinical aspect then closely resembles an infection with *Microsporum*.

Other objections have been raised by Langeron (1926) in his recent review of the classification of the Dermatophytes, among them being that the advances made in the knowledge of the structure and relationship of certain Dermatophytes have rendered Sabouraud's arrangement obsolete; and that the large number of new species described since his classification was elaborated makes the genera too large and ill-defined to be practicable.

Matruchot and Dassonville (1899, 1900) made a comparative study of the morphology of the Dermatophytes with a view to ascertaining their relationship to other fungi, and came to the conclusion that they are Gymnoascaceae which have lost the ability to produce asci, and, in fact, may be considered to represent conidial forms of some species of Gymnoascaceae. The evidence they put forward in support of this view is that in both a similar type of external spore, or aleuriospore, is formed; spindles are also found in the genus *Ctenomyces*; and all Gymnoascaceae have spiral hyphae as peridial ornaments, which are analogous to the spiral hyphae of certain species of small-spored Trichophyton (*T. asteroides*). They also considered that the spindles and aleuries are homologous structures and that all stages of transition from one to the other may be found.

Later (1901) they produced additional evidence in support of their theory by claiming to have produced lesions of a trichophytic nature in a dog by inoculation with *Ctenomyces serratus*, a species of the family of Gymnoascaceae, founded in 1880 by Eidam for that species, which he found growing on feathers. Matruchot and Dassonville found it also on feathers. On the standard Sabouraud media, cultures closely resemble those of *Trichophyton*. They then thought they had obtained definite proof of the correctness of their theory, by isolating *Eidamella spinosa* from ringworm of a dog. This fungus is a Gymnoascaceae which readily forms peridia in culture. The peridia contain bunches of pedicellate asci, each of which contains 8 ascospores. Cultures, however, never develop spindles or bunches of aleuries; and inoculation of dogs with cultures did not produce typical



ringworm lesions, but only slight epilation. Consequently, it is not certain that *Eidamella spinosa* was the causal fungus of the ringworm from which it was isolated, and it is probable that it may have been an accidental contamination. On the whole, Sabouraud's view in this connection is justified. He considers that the main evidence in support of the theory is the case of *Eidamella spinosa*, and that this is not reliable; and that, although the relationship of the Dermatophytes to the Gymnoascaceae is probable, it is not supported by sufficient evidence to permit of their being classed together.

Sabouraud's classification was generally accepted and was utilised by all subsequent workers until recently, irrespective of their opinion as to the systematic position of the Dermatophytes as a whole with respect to other fungi.

Ota and Langeron (1923) made the first attempt at a truly botanical classification of the Dermatophytes, and introduced a new classification based on mycological characters. From a general survey of the Dermatophytes they conclude that, although the old genera *Trichophyton*, *Microsporum*, *Achorion*, *Epidermophyton* and *Endodermophyton* can either not be retained at all, or only in a modified form, in a mycological classification, the numerous species do form a related group. In consequence of the aleuric form of spore being common to all the species, they place them in the Conidiosporaceae of Vuillemin's system, next to the Aleuriaceae, from which they differ in producing more complicated forms of spores in addition to the aleuries. They create the new sub-family Closterosporaceae for the Dermatophytes, the name being founded on the presence of the characteristic spindles.

The sub-family is divided into six genera: *Trichophyton*, *Sabouraudites*, *Epidermophyton*, *Grubyella*, *Bodinia* and *Endodermophyton*, of which *Trichophyton*, *Epidermophyton* and *Endodermophyton* are old genera in an emended form, and the other three are new.

Sub-fam. Closterosporaceae: generally with well-developed aleuries, and having in addition more complicated spores: arthrospores, spindles, and nodular organs.

(1) Genus *Trichophyton* Malmsten, 1848, O. & L. emend. 1923. Closterosporaceae with typical aleuries and arthrospores. Some species also have pedicellate chlamydospores. In this genus come the endothrix *Trichophyton*s of Sabouraud; the niveum group of small-spored ectothrix species; and all the large-spored ectothrix forms with downy cultures except *T. caninum*.

(2) Genus *Sabouraudites* n.g. O. & L. 1923. In this genus aleuries are as abundant as in *Trichophyton*, but, in addition, pectinate organs, spiral hyphae, spindles and nodular organs are formed. This is a large genus containing many species which were previously widely separated. It is divided into three sub-genera:

(a) *Aleurocloster*: species with both aleuries and spindles. Here are included most of the ectothrix gypseum *Trichophyton*s of Sabouraud, many *Microsporum*s, both human and animal, several of the animal *Achorion*s, and two species of *Epidermophyton*.

(b) *Closteramma*: species with aleuries, spindles and nodular organs. Contains 3 of the large cultured animal *Microsporum*s of Sabouraud, and the *Achorion* of the mouse—*A. quinckeanum*.



(c) *Aleuramma*: species with aleuries and nodular organs. Contains 2 Microsporums; 1 recently isolated *Trichophyton*, and the remainder of the small-spored gypsum Trichophyton of Sabouraud.

(3) Genus *Bodinia* n.g. O. & L. 1923. Aleuries rare, and, when present, rudimentary. Chains of arthrospores are usually present. Contains 1 *Achorion*; Sabouraud's 2 endothrix Trichophyton with glabrous cultures; and 1 other Trichophyton.

(4) Genus *Endodermophyton* Castellani, 1909. Differs from *Bodinia* in the fact that the hyphae forming arthrospores are less fragile and do not break up so easily. Contains 1 Trichophyton and the 2 Endodermophyton of Castellani.

(5) Genus *Grubyella* n.g. O. & L. 1923. Usually develop only arthrospores and rudimentary aleuries as in the two previous genera, but occasionally slightly more complicated organs are formed (e.g. external spores were observed in *A. schönleinii* by Sabouraud). Contains 1 Microsporium, *A. schönleinii*; and the large-spored ectothrix Trichophyton with faviform cultures of Sabouraud.

(6) Genus *Epidermophyton* Lang, 1879, O. & L. emend. 1923. Aleuries rare and rudimentary as in the three preceding genera, but numerous characteristic spindles are also formed. In the original form this genus contained five species, but of them only two were studied by Ota and Langeron, and of the two, one, *E. rubrum*, is placed in the genus *Sabouraudites*. There remains only the one species (*E. cruris* Castellani, 1905) in this genus, as the descriptions of the two species which were not studied by Ota and Langeron are insufficient to permit of their being classified.

There remains one species of Dermatophytes which resembles a Trichophyton in all respects except that, according to the authors by whom it was described, it forms peridia in culture, which generally contain asci and are of the type found in the Gymnoascaceae. For this species (*Trichophyton currii* Chalmers and Marshall, 1914) the new genus *Ateleothylax* of the Gymnoascaceae is founded.

Vuillemin (1925) introduced a new classification of the *Arthrosporales* in consequence of the problem presented by the Dermatophytes. In this he discards his former arrangement of the classes of Conidiosporaceae, and the aleurie is now regarded as characterising only a tribe, the Aleurismeae, which, because the species also form arthrospores, is removed from the Conidiosporaceae and placed in the Arthrosporaceae. The Dermatophytes, which Ota and Langeron (1923) placed in the Conidiosporaceae, in the new arrangement descend to the Arthrosporaceae, and are mostly united with the Mycodermas. The Dermatophytes developing spindles are united with *Fusoma*. This is criticised by Langeron (1926) on the grounds that in *Fusoma* the mycelium is very much reduced, and that the Dermatophytes are much more closely related to *Blastotrichum*, as has been shown by Ota and Langeron. Vuillemin retains the old "medical" genera in favour of those proposed by Ota and Langeron.

Grigoraki. As a result of his cytological investigation of the Dermatophytes, Grigoraki (1925) proposed a new botanical classification of the group. The basis of his classification rests on his conception of the pleomorphic degeneration of the spindles, which he regards as the essential reproductive structure, which gradually

degenerate in culture and ultimately grade into, and are replaced by, chlamydospores and aleuries. In view of this he considers that only "mother cultures" or primary cultures—*i.e.* cultures obtained directly by inoculation with the infected hairs or scales—are of value in determining the taxonomic characters of these fungi. According to this conception, starting with a species which when first isolated produces numerous spindles, as it is repeatedly sub-cultured it will undergo a gradual change in the organs developed, the spindles becoming less numerous, and more simple in structure; and the aleuries and chlamydospores correspondingly more numerous; until the stage of pleomorphism is reached, when aleuries are the only spores formed. He further considers that the species of Dermatophytes in which these forms of spores are developed may be arranged in a series of groups resembling definite stages in the progressive pleomorphic degeneration. Thus, the first group would have very numerous spindles and few or no aleuries or chlamydospores; and at the other end of the series would be a group with no spindles, but with very numerous aleuries, and resembling the third phase of pleomorphism.

Grigoraki makes three major groups of the Dermatophytes; the first two of which, in conformation with the theory of Matruchot and Dassonville, are classed in the Gymnoascaceae; and the third, which includes all the species in which only arthrospores are formed, except in rare cases where species have been kept a long time in culture, is placed in the Arthrosporaceae. Of those considered to be Gymnoascaceae, he agrees with Ota and Langeron in placing the two species *Eidamella spinosa* and *Trichophyton currii*, in which peridia have been described, in the genus *Ateleothylix* of the Gymnoascaceae. All the other species which correspond to the genera *Trichophyton* and *Sabouraudites* of Ota and Langeron are regarded as Gymnoascaceae which have lost the ability to produce perithecia, and are placed in a new family, Atelogympnoasceae, alongside the Gymnoascaceae.

This family is divided into five genera according to the principle of the gradual degeneration of the spindles. The five genera are:

(1) *Spiralia*, nearest in relationship to the Gymnoascaceae. Form some spindles, numerous large chlamydospores, very numerous aleuries, and, in a few days in culture, develop spiral hyphae which continue to be developed throughout life.

(2) *Closterosporia*. Spindles very numerous, forming the sole means of reproduction in primary cultures.

(3) *Closteraleurosporia*. Primary cultures have few spindles of variable form; chlamydospores and aleuries. Resemble species of *Closterosporia* in the first stage of pleomorphism.

(4) *Chlamydoaleurosporia*. No spindles, but only chlamydospores and aleuries. Resemble species of *Closterosporia* in second stage of pleomorphism.

(5) *Aleurosporia*. Primary cultures have neither spindles nor chlamydospores, but numerous aleuries. Resemble the third stage of pleomorphism of *Closterosporia*.

The species placed in the Arthrosporaceae include species of the genera *Bodinia*, *Endodermophyton*, and *Grubyella* of Ota and Langeron, and are all united in the one new genus *Arthrosporia*.

In a more recent paper Grigoraki (1927) states that when species of *Spiralia* are newly isolated, during the first four or five days in culture very numerous spindles are formed. These spindles rapidly give rise to large numbers of hyphae bearing chlamydospores and aleuries, and in older cultures the spindles are of secondary importance compared with the numbers of chlamydospores and aleuries. He considers that the abundant development of spindles in the early stages of cultures proves that these species should really be included in the genus *Closterosporia*.

Nannizzi (1926) considers that there is sufficient evidence to justify the classification of the Dermatophytes with the Gymnoascaceae. He arrived at this conclusion from a comparative study of a number of Dermatophytes and Gymnoascaceae; and because he found peridia-like organs are formed by some species of Dermatophytes when they are grown under suitable conditions on substances such as hair and feathers. He proposes a new classification of the Gymnoascaceae to include the Dermatophytes.

He divides the Gymnoascaceae into two sub-families: (1) Gymnoasceae Castellani and Chalmers, 1918; and (2) Atelogympnoasceae Grigoraki, 1924.

(1) Gymnoasceae is divided into two sections: (a) Eugymnoasceae Nannizzi, 1926, containing the genera *Myxotrichum* Kunze, 1823; *Gymnoascus* Baranetzky, 1872; *Ctenomyces* Eidam, 1880; *Amauroascus* Schroeter, 1893; *Arachniotus* Schroeter, 1893; and *Eidamella* Matruchot and Dassonville, 1901. The second section (b) Nothogymnoasceae Nannizzi, 1926, contains the genera *Myxotrichella* Sacc. 1892, and *Ateleothylax* Ota and Langeron, 1923. In the latter genus are placed the species of Dermatophytes in which he observed the development of the organs resembling peridia (*Trichophyton asteroides*, *T. radiolatum*, *T. denticulatum*, and *T. radians*), and the two species in which the development of peridia was previously described.

(2) Atelogympnoasceae Grigoraki, 1924. In this sub-family are placed all the Dermatophytes which formed the sub-family Closterosporeae of Ota and Langeron, except the four species which he transferred to the genus *Ateleothylax*. He retains Ota and Langeron's arrangement of genera, so that this sub-family contains the genera: *Trichophyton* Malmsten, 1848 (em. O. & L. 1923); *Sabouraudites* O. & L. 1923; *Bodinia* O. & L. 1923; *Endodermophyton* Castellani, 1909; *Grubyella* O. & L. 1923; and *Epidermophyton* Lang, 1879 (em. Ota and Langeron, 1923).

*Discussion.* At the present time Nannizzi's arrangement is the latest attempt at the classification of the Dermatophytes. The other classifications have been reviewed recently by Langeron (1926). Langeron objects to Sabouraud's system on the grounds which have already been mentioned; and it is generally accepted that this system is now obsolete; although it will, no doubt, continue to be used by medical workers on account of its convenience. A really scientific classification based on mycological characters is urgently needed if the group is not to develop into a hopeless multiplicity of ill-defined species arranged in large and even more ill-defined genera. Of the classifications proposed, that of Ota and Langeron seems to be the most satisfactory. It has the advantage of being based on a number of morphological characters of taxonomic value, aleuries, spindles, nodular organs,

and spiral hyphae, without attaching undue importance to any one organ or form of spore. The arrangement of the sub-family Closterosporae, and of the genera *Trichophyton* and *Sabouraudites* is good; but the differentiation of the genera *Bodinia*, *Endodermophyton* and *Grubyella* is very vague, and in the first two the relative fragility of the mycelium is of doubtful value in distinguishing two genera. *Grubyella* is also very ill-defined as it differs from *Bodinia* and *Endodermophyton* only in having, in addition to rudimentary aleuries and arthrospores, slightly more complicated spores, the occurrence of which is admittedly inconstant and sporadic.

Langeron (1926) criticises Grigoraki's proposed classification on a number of points. Primarily, he objects to it because it is based on the unproved theory of Matruchot and Dassonville regarding the relationship of the Dermatophytes and Gymnoascaceae; but also because several of the new genera are more or less duplications of those previously proposed by himself and Ota. He further criticises Grigoraki for violating Articles 44 and 45 of the International Code of Nomenclature, by not preserving the old generic names where possible; and for violating Article 38 by not indicating the type species of the new genera.

Although Nannizzi's work is undoubtedly very interesting and important, it is as yet unconfirmed for other strains and species, and, in the circumstances, the transference of all the Dermatophytes to the Gymnoascaceae seems to be premature. Further, even if it is accepted that the species in which peridia were formed are Gymnoascaceae, it is hardly justifiable to place them in the genus *Ateleothylax*, which was founded for a species forming true asci. At present there does not seem to be any advantage in placing all the remaining Dermatophytes in which peridia have not been found in the sub-family Atelogympnoasceae of the Gymnoascaceae, and it would probably be better to let them remain in the Closterosporae until further investigation reveals whether or not any of them are able to form peridia or asci under suitable conditions.

##### 5. TECHNIQUE.

*Obtaining and preserving infected material.* The general technique employed by Sabouraud (1910) is that generally used in studying the Dermatophytes. He recommends that the infected hairs and scales should be collected by means of a flamed forceps and placed between two previously flamed microscopical slides, and then wrapped in paper and kept until the material is required. If the hairs cannot be pulled out unbroken, the pieces should be as long as possible, in order that the structure of the fungus and its relation to the hair may be seen.

*Microscopical examination of infected material.* Direct microscopical examination of the hairs and scales may be carried out after clearing for a few seconds in 30 per cent. caustic potash (Sabouraud, 1910); or after long soaking in 5-7 per cent. caustic potash (Fox and Blaxall, 1896); or by mounting in 5-10 per cent. potash after clearing from grease by washing in ether, and examining during the clearing (Adamson, July, 1895). Langeron (1925) recommends mounting in chlorallactophenol, with or without the addition of sodium salicylate according to the density of the hairs to be examined. I have found this method much the best and most

convenient. It has the advantage that by sealing with Noyer's lanoline cement (Langeron, 1925) the preparations are rendered permanent for at least a couple of years. Sabouraud also recommends Berdal's method of heating the hairs or scales in formic acid, which has the advantage that it does not make them brittle.

*Permanent microscopical preparations.* Sabouraud makes permanent preparations by washing in water after clearing with caustic potash or formic acid, and mounting in glycerin and canada balsam respectively. For permanent stained preparations he recommends the following method: the hairs or scales are first freed from grease by soaking in chloroform, and are then placed in formic acid and heated to boiling-point for 2-3 minutes, after which they are well washed with distilled water. They are stained for 1 minute in "bleu de Sahli" prepared by mixing: distilled water 40 parts; saturated aqueous solution of methylene blue 24 parts; 5 per cent. solution of borax 16 parts, allowing to stand for 1 day and filtering. After staining, they are again washed in water; dehydrated with absolute alcohol; cleared in xylol; and mounted in balsam. I have found this method very satisfactory, although neither this nor any other method will penetrate into the depths of the hairs and stain the intra-pilar mycelium. Gram's stain may also be used, but is of no value beyond merely demonstrating the presence or not of a fungus. Stained preparations are useful for determining certain details of the structure of the invading fungi, but for studying the general structure of the fungi and their relation to the infected hairs unstained preparations are better.

*Obtaining cultures from infected material.* The method of obtaining cultures from infected material varies somewhat with the nature of the material. Infected hairs are dealt with as follows. An infected hair is placed on a flamed slide, and, by means of a sharp, flamed, scalpel, the root portion of it is cut into as many small pieces as possible. It is important to use only the root portion which was inside the follicle, as the extra-follicular portion of hairs is frequently contaminated with saprophytic fungi, especially in the case of animal hairs. The fragments are sown on agar slopes of one of Sabouraud's glucose or maltose media, by means of a flamed platinum needle. This is most easily accomplished by first touching the surface of the agar with the needle, to which the fragments of hair will then adhere on being touched with it, and are easily transferred from the slide to the tubes of medium. For each case about five tubes are inoculated in this manner, the fragments being spaced about one centimetre apart on the agar slopes. The large number of inoculations made in this way makes it probable that some at least of the resulting colonies will be pure cultures of the fungus. The tubes are kept at room temperature, which retards the growth of bacteria and many saprophytic fungi, but allows the Dermatophytes to grow freely. As soon as the colonies are sufficiently advanced they are sub-cultured on peptone "preserving medium"; and all subsequent sub-cultures are inoculated from the peptone cultures in order to reduce the danger of their undergoing pleomorphic changes to a minimum. In the case of herpes circinata, or ringworm of the glabrous skin, the expressed pus may be used for inoculation; or, if the vesicles are dry, fragments of scales are treated as in the case of hairs. Infected nails are dealt with by breaking up a shaving with sterile needles, or using



the filings made by means of a sterile nail-file. Favus cups are treated by smashing them between sterile slides, and inoculating with fragments from the interior region in the usual manner.

*Standard media.* The importance of the cultural aspect in Sabouraud's work made it essential that standard media should be used for growing the cultures described. Sabouraud introduced two types of medium: (1) "preserving medium" on which the cultures may be kept without undergoing pleomorphic changes; and (2) "proof media" on which the Dermatophytes grow extremely well and on which the different species develop the most differentiated and characteristic cultural forms. The "preserving medium" is composed of:

"Peptone granulée de Chassaing"	...	...	30 gm.
Distilled, or tap, water	...	...	1000 c.c.
Agar	...	...	18 gm.

The two "proof media" differ only in being prepared with different sugars, one being made with maltose and the other with glucose. The composition is:

Distilled, or tap, water	...	...	1000 c.c.
"Glucose massée de Chanut" or "Maltose brute de Chanut"	...	...	40 gm.
"Peptone granulée de Chassaing"	...	...	10 gm.
Agar	...	...	18 gm.

The preparation of these media requires special precautions, as overheating hydrolyses the sugars, and, as the reaction is acid, prolonged heating prevents the agar from forming a gel. Once the preparation is begun it must be carried right through without the agar ever becoming cool enough to gel; as if it does gel it will not solidify after being remelted. Sabouraud's directions for the preparation of these media are as follows, except that he advises filtering the medium, which is quite unnecessary. The ingredients are mixed and allowed to stand for half an hour. They are then placed in the autoclave, which is slowly allowed to reach 100° C., and, when a continuous jet of steam is issuing from the cock, the latter is turned off, and the gas turned down to about one-third of the full pressure. The temperature is allowed to rise slowly to 120° C., and the heat turned off immediately this temperature is registered. When the temperature has dropped to 100° C. the autoclave is opened and the medium at once filtered, if it is thought necessary, or distributed directly into tubes or flasks as required. Before they have had time to set, the tubes or flasks are replaced in the autoclave and the temperature again raised to 120° C. in exactly the same way as before, the heat being turned off immediately this temperature is reached, and the tubes or flasks removed as soon as it has dropped to 100° C.

Sabouraud recommends that large tubes, 18 cm. × 1.4 cm., should be used for cultures; and that, for studying the characteristic cultural forms, the cultures should be grown in Erlenmeyer flasks of about 500 c.c. capacity, with a layer about 1 cm. thick of medium at the bottom. Such flasks permit the cultures to develop freely and attain their maximum size without the form of the colony being destroyed by the edges coming in contact with the sides of the cultures vessel. Petri dishes

are not suitable for this purpose owing to the liability to contamination during the long period of growth, about 6 weeks, required for the maximum development of the Dermatophytes. Cultures are usually grown at room temperature, at which they grow readily and are less prone to pleomorphism than at higher temperatures. The Dermatophytes grow readily on practically all the usual mycological and bacteriological culture media.

*Hanging-drop cultures.* The structure of the mycelium is best studied in hanging-drop cultures made with one of Sabouraud's media prepared without the agar. For microscopical examination of the mycelium it may be mounted in cotton blue in lactophenol (Langeron, 1925), or stained with acid cotton blue (Langeron, 1925). I have obtained very good results by growing cultures on drops of one of Sabouraud's media (solid) placed on slides in moist Petri dishes, and staining with acid cotton blue; or, after fixation with Bouin or Duboscq-Brazil fixatives, with Delafield's haematoxylin. For temporary mounts, I found that mounting in about 50 per cent. glycerin, with sufficient congo red to colour it bright red, gave very good results.

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## EXPLANATION OF PLATES.

### PLATE I.

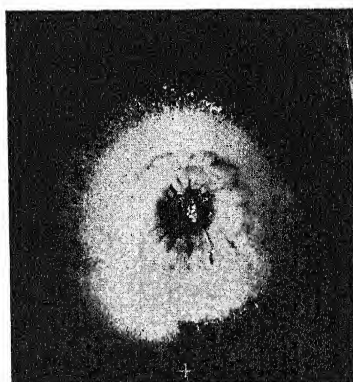
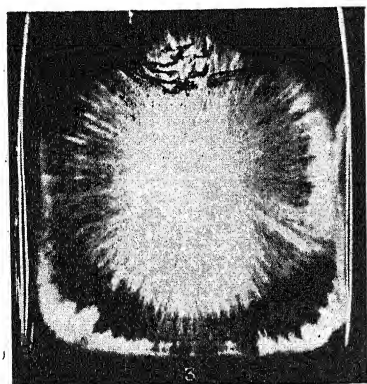
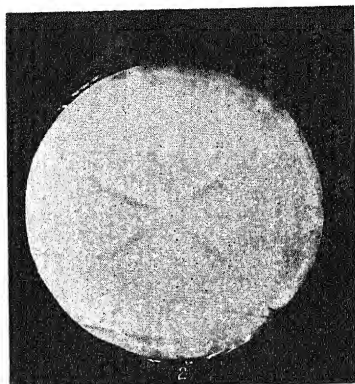
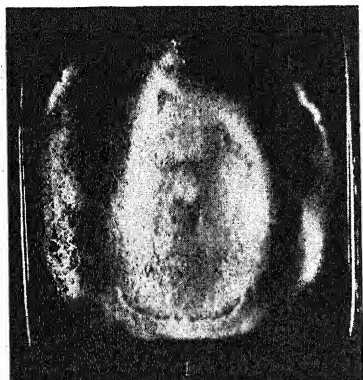
FIG. 1. *Microsporum felineum* Sab. FIG. 2. *M. audouinii* Gruby. FIG. 3. *M. lanosum* Sab. FIG. 4. *Trichophyton crateriforme* Sab. Cultures on glucose agar.

### PLATE II.

FIG. 5. *Trichophyton acuminatum* Sab. on glucose agar. FIG. 6. *T. sulfureum* Fox on glucose agar. FIG. 7 A. *T. crateriforme* Sab. tube culture on maltose agar. B. *Achorion gallinae* Sab. tube culture on peptone agar. FIG. 8 A. *Achorion schönleini* Lebert tube culture on peptone agar. B. *T. (faviform) album* Sab. tube culture on peptone agar.

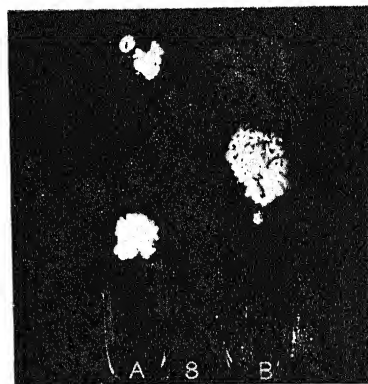
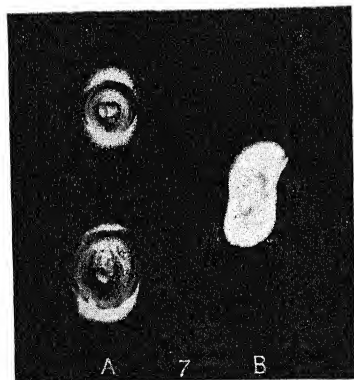
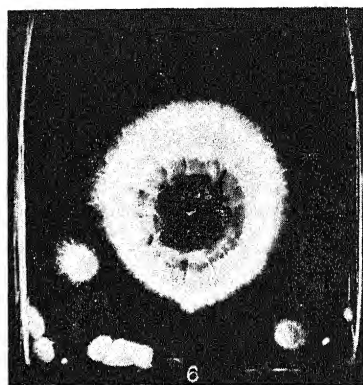
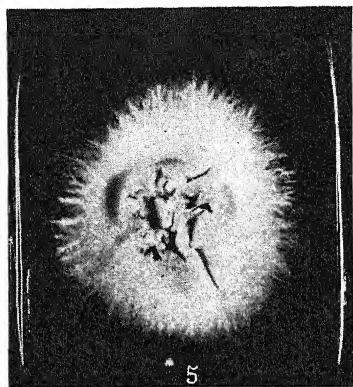
### PLATE III.

FIG. 9 A. *T. (gypseum) granulosum* Sab. tube culture on peptone agar. B. *T. (gypseum) radiolatum* Sab. tube culture on peptone agar. FIG. 10 A. *T. (gypseum) lacticolor* Sab. tube culture on peptone agar. B. *T. (niveum) radians* Sab. tube culture on peptone agar. FIG. 11 A. *T. radiolatum* normal form on peptone agar. B. *T. radiolatum* pleomorphic form on peptone agar. Both A and B are the same age. FIG. 12 A. *T. farinulentum* Sab. growing on a feather which was first washed in xylol to free it from fat. B. *T. farinulentum* growing on an untreated feather.



*C. Harpley, photo.*

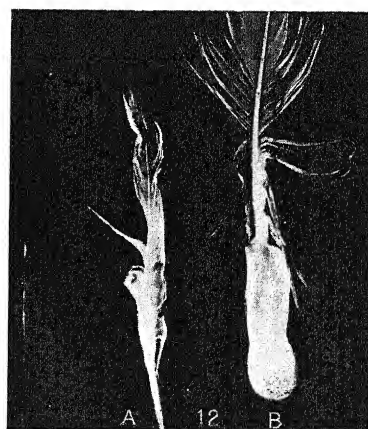
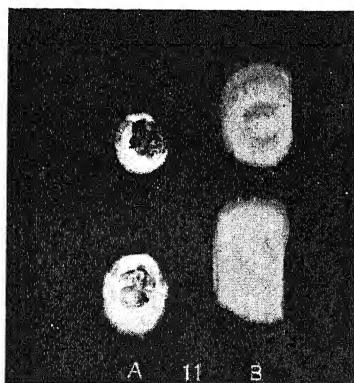
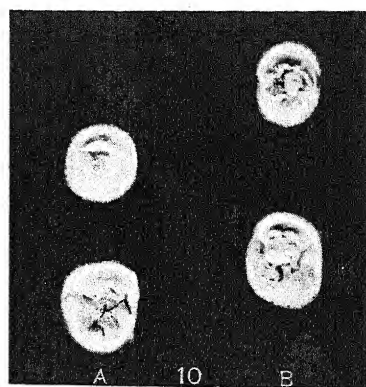
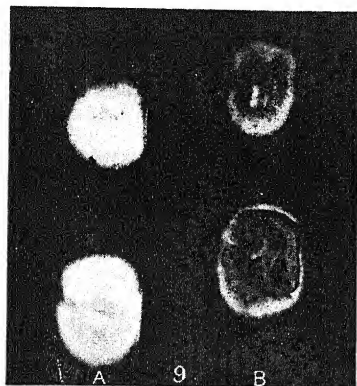
PLATE II



*C. Harpley, photo.*

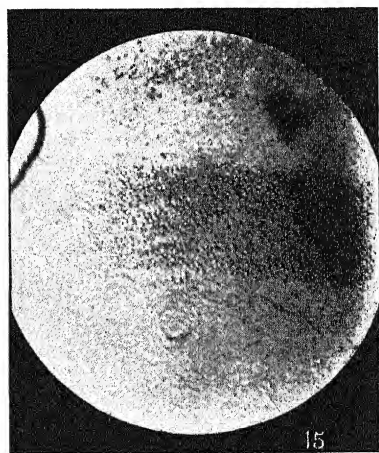
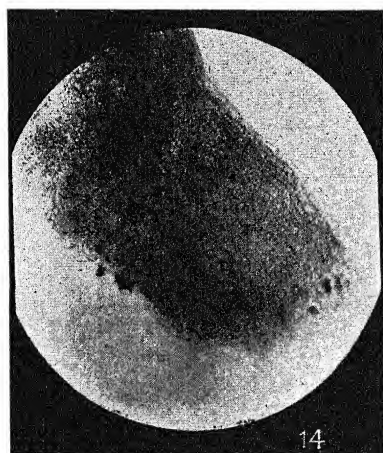
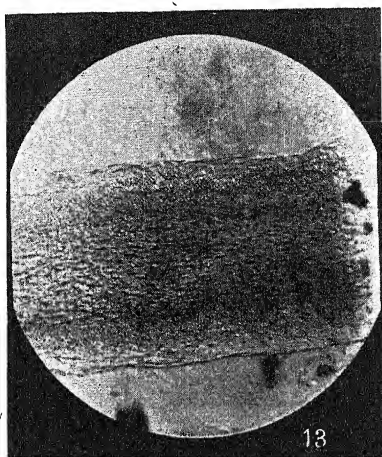


PLATE III

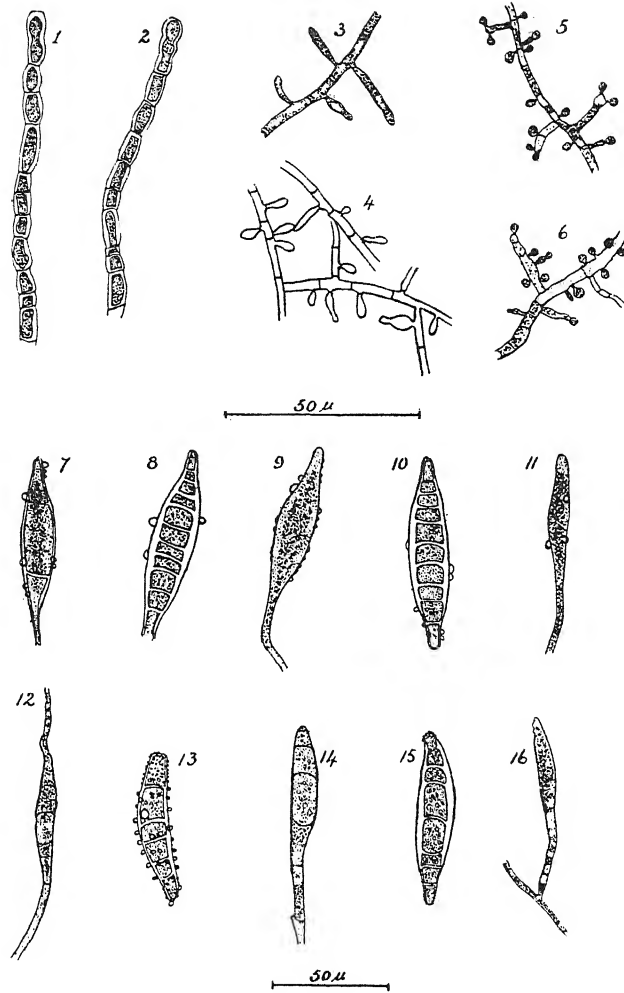


*C. Harpley, photo.*

PLATE IV

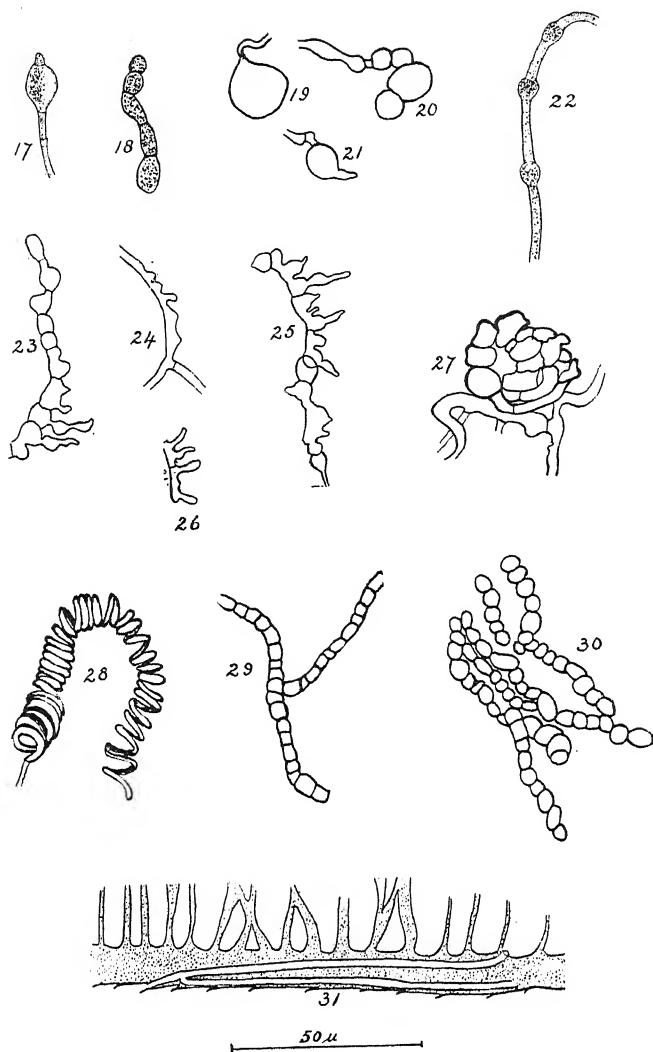


*C. Harpley, photo.*



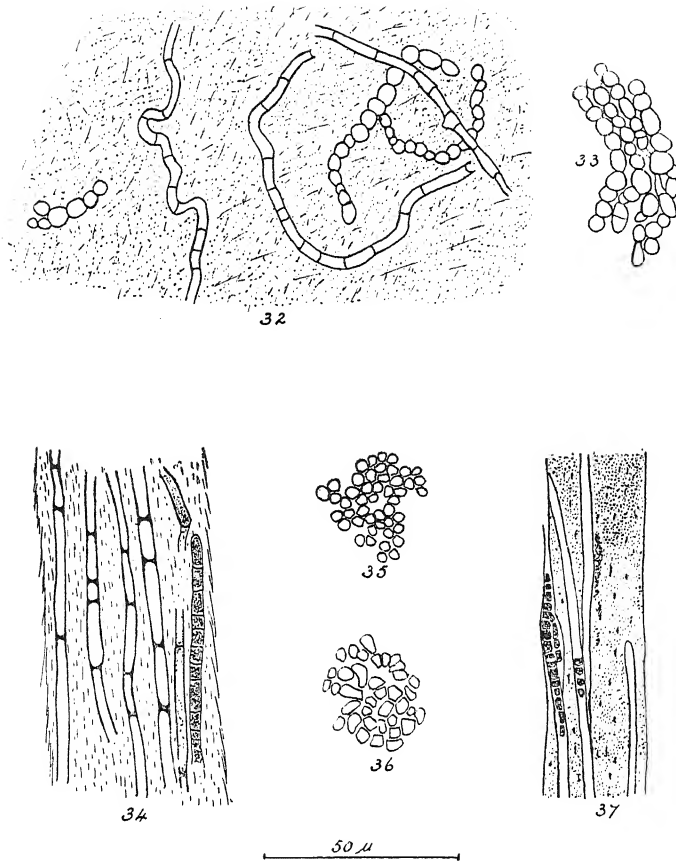
P Tate, del

PLATE VI



P. Tate, del.

PLATE VII



*P. Tate, del.*



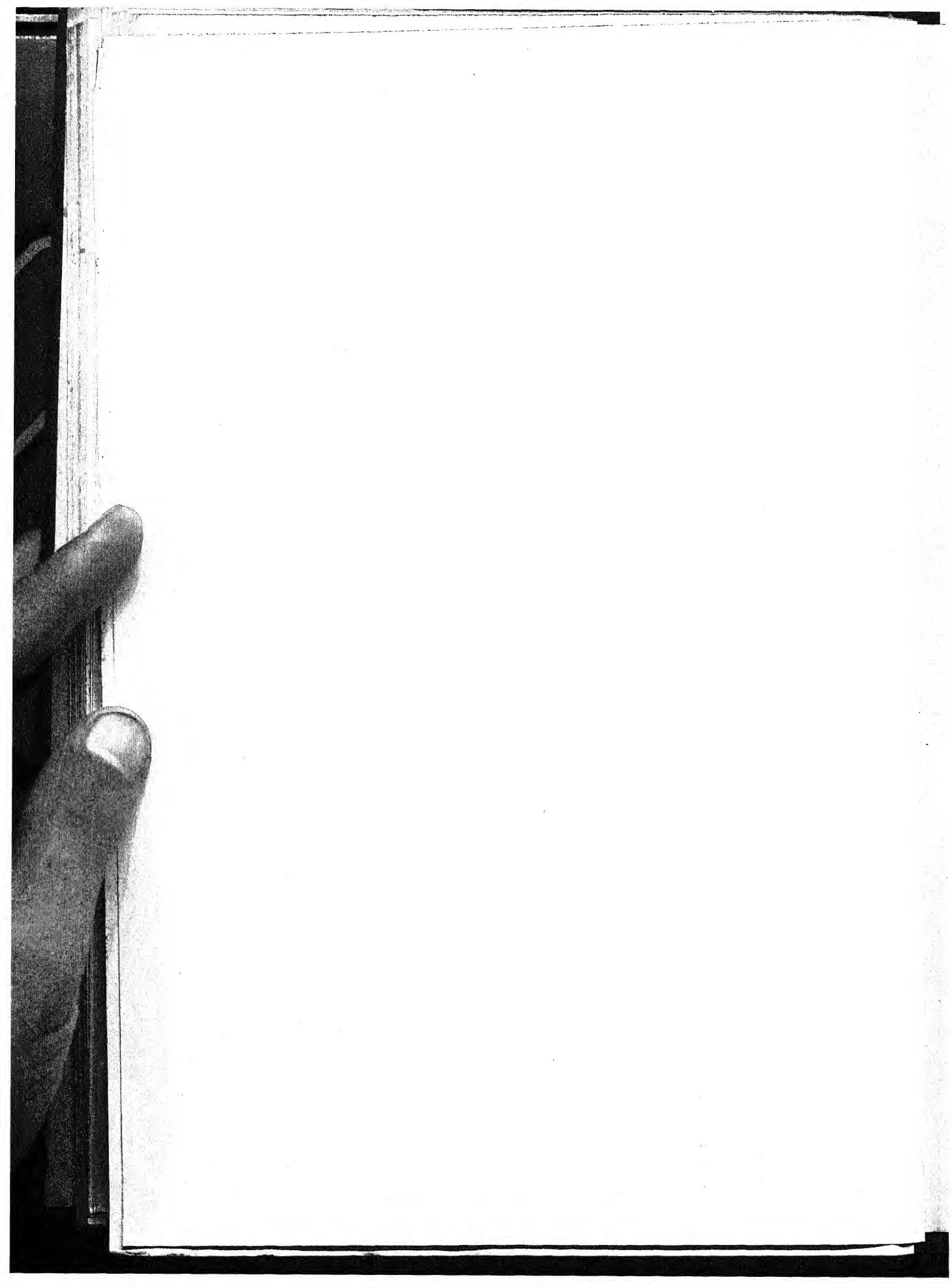


PLATE IV.

FIG. 13. Human hair infected with *Microsporum* (*M. audouini*). FIG. 14. Human hair infected with an endothrix Trichophyton (*T. grateriforme* Sab.). FIG. 15. Hair of a cow infected with an ectothrix Trichophyton.

PLATE V.

FIGS. 1, 2. Arthrospores. *Trichophyton tonsurans* (Malmsten). FIGS. 3, 4. Aleuries. *T. sulphureum* Fox. FIGS. 5, 6. Aleuries. *Sabouraudites radiolatus* (Sab.). FIGS. 7, 8, 9, 10, 11, 12. Various forms of "spindles" of *S. lanosus*. FIGS. 13, 14, 15, 16. "Spindles" of *S. felineus* (Sab.).

PLATE VI.

FIG. 17. Chlamydospore. *S. felineus*. FIGS. 18, 19, 20, 21. Chlamydospores of *Grubyella schönlleinii* (Lebert). FIG. 22. Racket-shaped hypha. *S. felineus*. FIGS. 23, 24, 25, 26. Denticulate hyphae (*M. velveticum* Sab.). FIG. 27. Nodular organ. *S. radiolatus*. FIG. 28. Spiral hypha. *S. radiolatus*. FIGS. 29, 30. Formation of arthrospores by intra-pilar hyphae of an endothrix Trichophyton: *T. tonsurans*. FIG. 31. Hypha of *S. lanosus* penetrating hair of guinea-pig.

PLATE VII.

FIG. 32. Ectothrix Trichophyton of Cow. Mycelium in epidermal scales. FIG. 33. Intra-pilar mycelium of the same ectothrix Trichophyton. FIG. 34. Intra-pilar mycelium of a *Microsporum* (*S. audouini*). FIGS. 35, 36. Portions of the spore sheath of *S. audouini* in the surface view. FIG. 37. Penetration of hair, intra-pilar mycelium, and formation of arthrospores, in hair of guinea-pig. (*S. lanosus*.)

# THE STRUCTURE OF PROTOPLASM

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*(Received October 23, 1928.)*

## INTRODUCTION.

SCIENCE offers few more baffling problems than that of the structure of the living substance of which we are made. Something of the arrangement of the coarser units of protoplasm can be seen, but there is as yet little unanimity of opinion on either the disposition or the significance of these units. Still less easy of solution is the interpretation of the finer structure of protoplasm, that which lies beyond the resolving powers of the microscope, and surely it must be this structure, rather than the visible one, which primarily determines the remarkable activities of living matter.

Before reviewing the work done on the structure of protoplasm, it will be well to make clear just what we are attempting to interpret. There is the possibility that we refer to the organisation of the cell as a whole, as does Harper (49), who says that "the structure of protoplasm is the structure of the cell." Cell organisation is intimately associated with, and is itself to a great extent determined by, the structure of the material which fills the cell, but the two are not identical, nor of the same category.

We could likewise limit ourselves to that which the microscope reveals, as did most of the earlier cytologists, but this also is not our intention. The scope of this paper stops short of cell anatomy and goes beyond visible protoplasmic structure to that finer construction whose unit is the molecule or a molecular aggregate.

The cytologist has to deal with essentially four domains in considering the structure of living matter; first, cell organisation, second, the visible microscopic configuration, third, the ultra-microscopic (colloidal) structure, and fourth, molecular orientation. There is a fifth domain, the arrangement of electrons, but of this we can say nothing in regard to protoplasm. No sharp line of demarcation exists between any of these realms. The second, third, and fourth are the province of this review.

The visible structure of protoplasm has been extensively studied, and figured with some degree of uniformity. The interpretation of the things seen has caused much controversy; nevertheless, we have a fairly accurate knowledge of the visible protoplasmic structure. More problematical is the nature of the invisible structure since the elucidation of it is entirely a matter of inference. The hypotheses evolved are inevitably of a highly speculative nature. To what extent is such speculation justified and to what extent is it helpful?

The opinions of men on the relative values of theory and experimentation are interesting and at times amusing. From one we have the statement that "a theory is to be trusted any time rather than an experiment," while another refuses to make a statement which is not supported by actual observation, "others may theorise." Both of these attitudes represent a state of mind which is bad. Speculation that does not rest on sound experimentation is dangerous, and experimental findings which are left uninterpreted are worthless. The great advances in science have been made by those who have seen beyond experimental results and have predicted what we now recognise as facts. It is the dreamers who find the key-stone to the arch of science. The rest of us merely lay the foundation, important, but more commonplace. Whether we call these dreamers fools or geniuses depends primarily on whether or not we agree with them. It was Kekulé who, dreaming on the top of a London omnibus, conceived of the benzene ring.

As necessary to knowledge as theorising is, we must see to it that our inferences are soundly drawn and that they rest upon experience.

The importance to life of structure, as contrasted with chemical constitution, has been frequently referred to in the literature. Jost<sup>(60)</sup>, having in mind the organism as a whole, says, "The mode of arrangement of the ultimate parts of the organism is of greater importance than the chemical nature of these parts." Henderson<sup>(56)</sup> expresses a mechanistic viewpoint in stating that "the organism is a Gibbs' system." Loeb<sup>(79)</sup> emphasises the necessity of "structure in the egg to begin with," otherwise, "no formation of a complicated organism is conceivable." And from an organic chemist<sup>(1)</sup> comes the statement that "a study merely of chemical constitution, however necessary, will carry us but a very little way in understanding even the simplest processes which take place in protoplasm, unless it be combined with a study of structure, and of the dynamics resulting from both."

#### PIONEER WORK.

Dujardin<sup>(23)</sup> and von Mohl<sup>(89)</sup>, among the earliest of students of the living substance, stated the physical properties of protoplasm with extraordinary accuracy. Their conclusions were uninfluenced by pre-existing theories and had the additional advantage of resting on observations of living material. Dujardin is credited with the discovery of protoplasm, which he called "sarcode." It is, however, very probable that those still earlier investigators—that inimitable observer and portrait painter, Rösel von Rosenhof, who saw and drew amoeba in 1755; O. F. Mueller, who described living amoebae in 1773, and Ehrenberg, pioneer protozoologist—must surely have seen the living substance itself; but to Dujardin belongs apparently the sole credit of realising that the visible stuff he saw is what makes a rhizopod alive. Dujardin's careful description of protoplasm is to-day, nearly a century later, as accurate as any we can now give. He<sup>(23)</sup> said, "Je propose de nommer ainsi ce que d'autres observateurs ont appelé une gelée vivante, cette substance glutineuse, diaphane, insoluble dans l'eau, se contractant en masses globuleuses, s'attachant aux aiguilles de dissection, et se laissant étirer comme du mucus, enfin se trouvant dans tous les animaux inférieurs interposée aux autres éléments de structure."

Von Mohl<sup>(89)</sup>, who gave us the name "protoplasm," characterised the living substratum in similar terms. He described it as "niemals einen klaren wässrigen Zellsaft... sondern... eine Zähflüssige... Masse."

It is significant that both Dujardin and von Mohl recognised the high consistency of protoplasm and its jelly-like nature, a fact which we shall have occasion to emphasise later.

#### VITAL UNITS.

That protoplasm is a heterogeneous substance and therefore possesses structure, if none other than the structure of a conglomerate, was recognised by all earlier workers. The lifeless nature of some of the included bodies, such as fat droplets and starch grains, was also appreciated. This led to attempts to differentiate between the living and the non-living in protoplasm. Hanstein<sup>(46)</sup> distinguished between the active and living protoplasm and the passive and lifeless "metaplasm." Sachs<sup>(100)</sup> termed the former "energid" and the latter "energid products." The term "metaplastic" (or "paraplastic") still persists for the metabolic products which are generally recognised as lifeless.

Other biologists have wandered far into the philosophical and speculative field by postulating special vital bodies which give to protoplasm the properties of life. Buffon<sup>(10)</sup>, Verworn<sup>(124)</sup> and others have conceived of gigantic molecules termed "biogens" which are the life-giving elements of protoplasm, the rest of the material being presumably non-living. Spencer<sup>(117)</sup> postulated "physiological units," and Altmann<sup>(2)</sup>, "bioblasts." In this same category, though referring more particularly to heredity determining characters, belong the "gemmules" of Darwin, the "pangens" of de Vries, the "plastidules" of Haeckel, the "biophores" of Weismann and the "genes" of modern geneticists.

Nägeli's<sup>(91)</sup> "idioplasm" theory, while speculative like the others, has the advantage of resting on a chemical foundation which is in harmony with certain present known facts. He accounted for hereditary traits by specific molecular orientations. This led to the conception of elementary units of structure, aggregations of molecules, which Nägeli termed "micellae" and which were destined to play a prominent rôle in subsequent theories of protoplasmic and colloidal structure.

These theories are highly speculative, yet some are not far removed from modern physical-chemical viewpoints. Numerous physiologists have expressed the belief that a definite substance or group of substances represents the ultimate living material. Leathes<sup>(68, 69)</sup> states that proteins are generally considered the most important components of protoplasm. Pauli<sup>(95)</sup> has said, "There can be no doubt as to the central position of the proteins in the organisation of living matter. They alone display the specific properties of life. Distinctions observed not only between different kinds of organisms but often between individuals of the same kind, reappear on chemical investigations as variations in the respective proteins. The proteins are capable of showing a diversity and fine gradation both in chemical structure and in physical modification to an extent which is lacking in any other class of substances." There has prevailed among many workers the



opinion that a "protein complex is the ultimate living substance"<sup>(114)</sup>. The constituents of this complex are probably of the nature of enzymes.

Any discussion on the existence of an ultimate, fundamental, vital substance inevitably leads to the question, now of long standing, Is it possible to distinguish between the living and the non-living in protoplasm? That the living molecule possesses some property not common to other molecules is the contention of one group, while others maintain that no such distinction exists, and that the living substance is alive because it is an organised *system*, the component parts of which are lifeless when considered individually, but which, in the associated co-ordinated state, produce life.

Hopkins<sup>(58)</sup> says, "We can scarcely speak at all of living matter in the cell. At any rate we cannot. . . speak of the cell life as being associated with any particular type of molecule. Its life is the expression of a particular dynamic equilibrium which obtains in a polyphasic system. Life. . . is a property of the cell as a whole." One must, of course, agree with Hopkins' further remark that "the integrity of metabolic life of a liver cell is as much dependent on the existence of a metaplasmic glycogen, however small in amount, as upon the existence of the nuclear material itself." So also is the running locomotive dependent upon its fuel of coal and water, but the ultimate mechanism is a structure of steel. Living protoplasm has its fuel and a mechanism for converting the fuel into potential energy.

Wilson<sup>(131)</sup> agrees with Hopkins in saying, "The term protoplasm does not designate a single substance but is a collective name for the sum-total of the active components that co-operate in the work of a complex system; and life is the sum-total of the activities of that system." Sharp<sup>(115)</sup> believes it to be a fundamental fallacy to attribute the properties of a system to one or more of its constituent elements. Mast<sup>(85)</sup> is equally firm in this belief. He states, "If, then, protoplasm is defined as living substance, its structure must involve the cell as a whole, not. . . this portion or that portion. . . ."

The belief in a basic substance carries with it the concept that protoplasm is a living *system*<sup>(114)</sup>.

The distinction between living and non-living is more readily grasped if one considers a larger system, such as a plant. Here it is clear that the waxy cuticle of the leaves, the stored food in the vessels, the latex, and like matter, are absolutely necessary for the well-being of the plant, yet none are regarded as alive. Just so does protoplasm contain its non-living constituents, its nutrient matter, its internal environment.

#### THE GRANULAR THEORY.

The early theories of protoplasmic structure are so well known to cytologists and have been so thoroughly reviewed in a number of publications<sup>(21, 25, 46, 115, 130)</sup> that only a brief outline of them will be given here.

The picture which living protoplasm often presents when viewed through the microscope is that of a glassy substance in which many tiny particles are suspended. Even more pronounced does this granular appearance become when the material is "fixed," *i.e.* killed with the reagents used in cytological technique (formaldehyde,

alcohol, chromic acid, etc.). These facts led to the hypothesis, sponsored chiefly by Altmann<sup>(2)</sup>, that the structure of protoplasm is a granular one. The granules were christened "microsomes" by Hanstein in 1882 and, as Bütschli<sup>(11)</sup> remarks, they, therefore, obtained the right of entry among the privileged and recognised units of protoplasmic structure, for "anything that is called by a Greek name at once seems to many people to be much better known, and as something which must be definitely reckoned with."

Altmann carried his speculations rather far when he stated that the granules were comparable to free-living bacteria. Thus the cell becomes a colony of minute organisms, the granules or bioblasts. While such a conclusion is improbable yet it is not long since certain of these same granules, the mitochondria, were regarded as bacteria, and to-day we recognise that plastids arise only from the division of pre-existing granules, *i.e.* that they have a certain autonomy apart from the cell. The scurrying about of the rod-shaped mitochondria in a living fibroblast cell in tissue-culture leads to the opinion that these inclusions get along quite independently. Wilson<sup>(129)</sup> expresses a similar opinion.

The criticism that granules in fixed material are artifacts cannot be brought against them as structural units for they are there in abundance in living material. The problem has not to do with their existence but with their rôle in life. Heilbrunn<sup>(55)</sup>, whose work has been entirely on living matter, states that "the interior protoplasm of the sea-urchin egg is a suspension of visible granules and its colloidal behaviour should be interpreted on this basis."

The two chief criticisms of the granular hypothesis of protoplasmic structure are, first, that it is rather a "motley collection which is here brought together under the head of granules." There are the ultra-microscopic particles about which we know nothing except that they are there, the minute visible particles which are mere specks, alveoli, "alveolar spheres" (of yolk), vacuoles of all gradations in size, oil globules, starch grains, plastids, secretory granules, mitochondrial spheres, rods and threads, chromatin granules, etc.

The second criticism of the granular hypothesis is the difficulty of doing anything with it as a basis of interpretation of physiological processes.

#### FIBRILLAR THEORIES.

There has long persisted in the minds of biologists the thought that there must exist a continuous framework of some sort as the structural background of protoplasmic behaviour. Life in a solution of isolated units, no matter how complex the mixture, is inconceivable. This philosophical conception is well supported by actual observations on fixed and stained material.

The advent of cytological technique, between the years 1870 and 1890, brought a great impetus to the study of protoplasmic structure, and yielded much of value even though many of the ideas formulated at the time and since have had to be discarded. During these years numerous hypotheses on the nature of the structural background of living matter were developed. The living substance was viewed variously as an entanglement of fibres, a three-dimensional net, and as a sponge.

All of these classical theories of protoplasmic structure which are based on the concept of continuity in structure need to be modified but little to become essentially the equivalent one of the other. There are two other classical theories, that of Bütschli who compared protoplasm to an emulsion or (erroneously) to a foam, and that of Nägeli who believed the structural unit of protoplasm to be an aggregation of molecules, a micella. These theories, though they have something in common with the fibrillar ones, stand quite apart, since they represent the first attempts to interpret protoplasmic structure on a colloidal basis. They will, therefore, be considered separately from the purely cytological theories.

*The filar theory.* The so-called filar theory, supported by Flemming<sup>(33)</sup> and others, ascribed to protoplasm the structure of an entanglement of fibrils. Flemming elevated these fibrillae, as did Altmann his granules, above the lowly station of mere structural units and viewed them as the seat of the energies on which life depends.

That protoplasm often shows a fibrous structure when fixed and stained is certainly true. The drawings of Flemming<sup>(32)</sup> of connective tissue, and of Heidenhain<sup>(51)</sup> of muscle and spinal ganglion cells, clearly depict a fibrillar structure. Here the fibrillae are to be regarded as features of the protoplasm itself rather than as cell organs, though naturally such a distinction cannot always be sharply drawn.

Fibres of a larger sort, which assume the individuality of distinct cell and tissue structures, are of many types and functions. The continuation in the cell of such external fibres as cilia<sup>(130)</sup> and the neurofibrils of the neuromotor apparatus of certain protozoa<sup>(122)</sup> are examples of fibrous cell organs.

The fibrous structural units of connective tissue are of colloidal dimensions. Ettisch<sup>(26)</sup>, with the aid of dark-field illumination, finds the construction of sinew to be that of an aggregation of colloidal fibres.

The filar theory of protoplasmic structure, by some discarded with its contemporaries, has been resurrected in another form, with the linear molecule as the unit. Protoplasm, like its non-living relative, gelatine, is believed to possess a structure crudely comparable to a "brush heap," or disorderly pile of brushwood, of which the units are chain molecules<sup>(110)</sup>. The frequency with which fibres are to be seen in cells—spindle fibres, neurofibrils, structural fibrillae, etc.—suggests the presence of finer linear units whose orientation leads to the formation of these grosser fibres.

*The reticular theory.* Linear structural units may be orientated so as to form an entanglement such as exists in a brush heap, or they may be more symmetrically arranged and form a three-dimensional net. Earlier controversies often centred on the question whether the protoplasmic fibres are discontinuous or whether they anastomose to form a net or reticulum. That they are, or maybe, separate units was the belief of Flemming and Heidenhain, which viewpoint is now supported by Fauré-Fremiet<sup>(27)</sup> and other modern workers.

That a network results was the opinion of Frommann<sup>(40)</sup> who, perhaps, deserves the credit of being the first (1865) to advance a concrete hypothesis of protoplasmic structure. A few years later (1870) Kupffer<sup>(64)</sup> described the protoplasm of living

follicle cells of the egg of *Ascidia canina* as having a perfect reticular structure. The meshes of the three-dimensional net are from  $\frac{1}{2}$  to  $2\mu$  in size. The morphological and physiological nature of the reticulum was interpreted in various ways. Leydig<sup>(77)</sup> regarded it as an open framework, like a sponge, and only of structural significance. The real living matter, the "hyaloplasm" or "enchylema," he considered to be the substance which bathed the purely anatomical framework. Others have viewed the sponge framework itself as the primary living substance.

Modern cytological hypotheses of nuclear and chromosome structure, of granules on a linin reticulum, are similar to the old reticular theories of protoplasmic structure<sup>(12, 113)</sup>.

Gasser and Hill<sup>(44)</sup> have interpreted muscle action on the basis of a viscous-elastic system, consisting of an elastic network, through the minute passages of which a viscous liquid is forced when constraint is applied to the system.

The objections which have been lodged against the reticular hypothesis are that this structure is not typical of the living substance, but is a post-mortem thing, and that a permanent and rigid framework will not permit flowing, yet protoplasm certainly flows. The criticism that reticular protoplasm is the result of post-mortem changes will be considered later under the general head of fixation and artifacts. The second criticism is justified only when the net or sponge is regarded as a fixed arrangement of parts. A state of permanent structural fixity never obtains in protoplasm, nor even in cells with relatively constant anatomical features such as some of the protozoa possess, for, even here, the cell configuration is completely broken down at mitosis and reconstructed later.

The weakness of the old hypotheses lies in the attempt to formulate too rigid and too coarse a structure. Protoplasm is alive, dynamic, and ever changing. The living substance undoubtedly possesses a definite and characteristic structure, but this structure is as labile, as capable of change and readjustment, as life itself.

#### THE ALVEOLAR THEORY.

Bütschli's<sup>(11)</sup> classical theory of protoplasmic structure will be twice discussed in this review, since it holds a dual position, first, among the classical cytological theories now under consideration, and second, as one of the first attempts to view protoplasmic structure in the light of colloidal physics.

Robert Hooke<sup>(57)</sup> in 1665 described the pith of a feather as a kind of solid froth. Bütschli regarded protoplasm as also having such a structure which he characterised as "foam-like, alveolar or honeycombed."

Andrews<sup>(3)</sup> has given an excellent review of Bütschli's theory.

Taylor<sup>(122)</sup> has described alveolar protoplasm in *Euplotes* where this structure also characterises the macronucleus<sup>(113)</sup>. The nuclei of amphibian red blood cells are an emulsion<sup>(113)</sup>. Comandon<sup>(19)</sup> has cinematographed *Triton* erythrocytes and shown most strikingly that the nuclei are alveolar. A pseudo-alveolar structure results when cells become packed with yolk or fat<sup>(113, 130)</sup>, as Fell and Andrews<sup>(28)</sup> have described in fibroblast-like cells.

Bütschli interpreted the frequently observed reticulum in protoplasm as a mesh-work formed by the fluid lamellae of an emulsion or "foam." In this he was

right, since all of the classical reticular and sponge hypotheses of protoplasmic structure are but interpretations of the picture which both the living and the fixed and coagulated emulsion often presents.

The alveolar structure is but one of the numerous configurations which the living emulsion can assume.

Emulsion globules under pressure become hexagonal in cross section. Their three-dimensional shape may be either that of the rhombic dodecahedron or orthic tetrakaidecahedron, forms generally acknowledged to be the shape that cells in mass and under pressure assume. This belief is based on the thorough investigations of Kelvin<sup>(62)</sup>, Lewis<sup>(71, 72)</sup> and others. The chemical constitution of the alveolar contents varies but is essentially vacuolar sap<sup>(113)</sup>. Chamberlain<sup>(12)</sup> regards all forms of alveoli as simply vacuoles under different physical conditions.

#### THE MEMBRANE.

The fact that a cell part arose from the basic ground-substance is no proof that it is of the same chemical constitution and physical nature as the material from which it came. Thus, a starch grain is a different sort of thing than the pyrenoid which produced it, and a cellulose wall is of quite different material than the cytoplasm which secreted it.

Those cell parts which can be regarded as cell organs and therefore as living basic matter, such as nucleus, chromosomes and membrane, are physical systems fundamentally identical to the cytoplasmic ground-substance. While the chemical constitution of the nucleus, chromosomes and membrane differs from that of the cytoplasm (this is not so marked in the case of the membrane), the physical properties, and therefore the ultra-microscopic structure, of all are essentially the same. The microscopically visible structure of the nucleus is in some cases identical to that of the cytoplasm.

A consideration of other cell parts, the smaller inclusions, brings us to that uncertain and controversial distinction between the living and the non-living. A chloroplast, that is, the substance chlorophyll, may be looked upon as a living system. Chloroplasts reproduce themselves and reproduction is our one infallible criterion of the living state. Wilson<sup>(129)</sup> accepts the probability that many of the smaller protoplasmic plastids have a persistent identity and perpetuate themselves by multiplication. There is no very serious objection to be raised against the idea that chloroplasts, pyrenoids, mitochondria and the like are living cell parts, until we look further.

Chlorophyll, haemoglobin, enzymes, vitamins and filterable viruses have much in common. Are they to be regarded as living or non-living systems?

That the human red blood cell is a living system many doubt and we now have evidence that filterable viruses are not living. What then about chlorophyll? The question is unanswerable.

The field of this review, as indicated by its title, "The structure of protoplasm," would preclude a consideration of the structure of a starch grain, or a calcium oxalate crystal or the cellulose wall, but chloroplasts we shall regard as within our



ken, so also mitochondria whose vital properties one cannot doubt, though of their structure nothing can be said, they are too small.

No cell part has received more attention than the external layer. Overton (94) regarded it as ordinarily fatty in nature. Recent work indicates that the outermost surface of protoplasts is coated with fat. Clowes (13) has drawn an analogy between the semi-permeable properties of the protoplasmic membrane and the behaviour of emulsions, thus implying that the cell surface is an emulsion of oil and an aqueous solution. That the protoplasmic membrane is a di- or polyphase system is the opinion of Nathansohn (92), who compared the surface layer to a mosaic.

Investigations on the physical properties of the membrane have thrown some light on the nature of the cell surface layer. But we must stop here for a moment and consider just what we have in mind under the term membrane. The cellulose wall of plants is, especially in German articles, sometimes referred to as membrane. This has led to confusion, since it is not a cellulose wall which workers usually have in mind when speaking of the protoplasmic membrane. The other extreme is represented by a monomolecular layer which Fricke (39) thinks represents the protoplasmic membrane of erythrocytes. Mudd and Mudd (90) do not agree, nor do others (111). A thin oil layer may well exist—its presence on cell surfaces may be very general—but the membrane to which cell anatomists refer is a morphological thing, optically visible under favourable conditions.

There is much difference of opinion on the identity of a protoplasmic layer at the surface of protoplasts. Fischer (31) says there are no membranes around cells. Chambers (13) grants that the surface layer is more dense, a gel, but believes that it passes gradually into the sol condition of the interior.

The technique of microdissection has clearly demonstrated that the membrane delimiting not only the outer surface of the protoplast but also certain cell inclusions is an anatomical structure.

Vonwiller (125), having shown by vital staining that amoeba possesses a definite surface membrane, thought the same to be true of the nucleus, though his method did not permit him to prove it. Micrurgy has shown Vonwiller's belief to be true. The surface layer of an isolated and coagulated nucleus of amoeba may be lifted off with microneedles, stretched and torn (107). It resembles a delicate veil when stretched. Howland (59) has demonstrated the presence of a similar morphological membrane on the surface of *Amoeba verrucosa*. Of importance, as an indicator of structure, is the elastic quality of the membrane. This property has been demonstrated by several workers (13, 107) and is used by Krasnosselsky-Maximow (62\*) to explain differences in plasmolytic concentrations of the cell.

We cannot enter here into the long and now historical discussion on the chemical constitution of the protoplasmic membrane, except to refer briefly to what appears to be the generally accepted view, namely, that the superficial layer is coated with a thin oil film, while the body of the membrane is essentially protein in nature. The work of Mudd and Mudd (90) indicates the presence of an outer oil coating on erythrocytes and certain bacteria (electric conductivity suggests the same), while the substantial and highly elastic nature of the erythrocyte pellicle points clearly

to a protein constitution of the body portion of the membrane<sup>(111)</sup>. Rigidity and elasticity, so characteristic of the protoplasmic surface layer, are not properties of oil films and emulsions.

Like protoplasm in general, the membrane is capable of change in its physical properties. It may become quite liquid<sup>(107)</sup>. This is true of even so substantial a pellicle as that of the protozoan *Euplotes*<sup>(112)</sup>.

Taylor<sup>(123)</sup> has shown the "sol-gel reversibility" of the cytoplasmic membrane to be an important factor in the functioning of contractile vacuoles.

In conclusion, we can say that the morphological membrane of protoplasts is in every sense protoplasm. It differs rather in the relative proportions of its constituents than in their kind. It is more dense than the interior protoplasm, due to a closer interlocking of the structural units. It does, however, appear that the membrane is usually coated with a thin film of oil. A consideration of the nature of the structural units will close this review.

#### THE NUCLEUS.

Nucleoplasm is protoplasm, that is, it is living material; consequently, the remarks so far made on the structure of protoplasm should, and do, apply to the nuclear substance. Nuclear structure has, however, had a long chapter for itself in the history of the physical properties of living matter.

The literature forces us at the outset to consider the question of the very existence of the thing we are trying to interpret—Have nuclei actually a structure? The only possible answer to this question is "Yes." It should be borne in mind that only the structure of the so-called "resting" nucleus is under consideration.

Flemming<sup>(34)</sup> and Strasburger<sup>(120)</sup> more than forty years ago described structure in the living nuclei both of plants and animals. Flemming<sup>(34, 130)</sup> figured structure in the living nuclei of salamander larvae. Similar observations have often been made since. Wilson<sup>(130)</sup> gives a partial historical review of this work.

Much of the work on nuclear structure has been done by the usual cytological methods of fixing and staining. This has raised the question of the significance of the structures seen, whether they have any bearing whatever on the arrangement of units in the living stuff. While these questions are, in the main, justified, they do not necessarily invalidate the observations made, for Strasburger was able to follow almost the entire course of mitosis in the living objects, which at the same time showed a certain amount of structure in the "resting" nuclei (see especially Strasburger's figures on *Tradescantia* in his *Zellbildung und Zelltheilung*).

Earlier workers on the structure of nuclei found a framework which they regarded as essentially the same type of net or sponge reticulum as is to be seen in cytoplasm. This view is still held by modern cytologists who regard the framework as a "linin" thread upon which "chromatin" granules are scattered. Heidenhain<sup>(51, 130)</sup> figures the chromatin network of nuclei from the crypts of Lieberkühn in the salamander, showing chromatin masses, a few linin threads, and the filling-in ground-substance or nuclear sap.

Recent workers have viewed the linin thread as the lamellae of an alveolar

(emulsion) structure, the chromatin granules as the nodal points of the inter-alveolar substance, and the ground material or sap as the alveolar contents<sup>(113)</sup>. Chamberlain<sup>(112)</sup> makes this clear in stating that "there are no such structures as chromomeres upon a linen ribbon." He regards the nucleus as of the same "vacuolated" (*i.e.* alveolar) nature as the cytoplasm, and he adds that this is to be expected if the one was derived phylogenetically from the other.

That certain nuclei are alveolar in the strict Bütschlian sense has been shown to be true for two quite distinct types of nuclei<sup>(113)</sup>. The long macronucleus of *Euplotes* is symmetrically alveolar. Comandon<sup>(119)</sup> has illustrated alveolar structure in *Triton* blood-cell nuclei. The nuclei of *Cryptobranchus* erythrocytes are also an emulsion of alveoli closely arranged in a dispersion medium. The belief is expressed<sup>(113)</sup> that the alveoli are of the nature of vacuoles, and their contents, therefore, to be regarded as essentially non-living, while the continuous phase of the nuclear emulsion is the fundamental living material.

There is considerable difference of opinion in regard to the presence of a visible structure in living nuclei. Chambers<sup>(115)</sup> finds no structure in the nuclei of metazoa. Lewis<sup>(76)</sup> is of the same opinion. The ultra-microscopic studies of Strangeways and Canti<sup>(119)</sup> have revealed no nuclear structure in tissue-culture cells.

Structures in the dividing cell have only an indirect bearing on our problem. Spireme and like mitotic features are temporary, but they suggest a pre-existing architecture.

The "resting" nucleus of amoeba is generally regarded as possessing a definite and discernible structure in the living state.

Mast<sup>(84)</sup> pictures structure (peripheral particles) in the nucleus of *Amoeba proteus*. It also appears that the nucleus of *Amoeba bigemma* has a visible structure consisting of an outer hyaline zone and a central granular mass.

Scarth<sup>(104)</sup> has recently described a microscopically visible structure in plant-cell nuclei. The optical character of the nucleus ranges from apparent homogeneity in *Symphoricarpus* and *Spirogyra* through a fine grained heterogeneity in *Elodea*, to a coarsely mottled appearance in *Tradescantia*. The apparently homogeneous plasm of the *Symphoricarpus* nucleus reveals the presence of a firmer portion when it is rapidly ejected from the cell through a small puncture.

Wilson<sup>(130)</sup> summarises the work on nuclear structure with these words: "The strongest evidence of the pre-existence of some kind of nuclear framework is...the gradual formation from it...of the spireme thread,...the apparent absence of structure so often observed in living nuclei is deceptive..."

#### CHROMOSOMES.

Cytological literature contains numerous interesting references to chromosomal structure in fixed material. Certain of these descriptions fit in well with the general idea that protoplasm, in its coarser microscopically visible structure, is an emulsion.

Two of the earliest papers on the subject are those of Baranetzky<sup>(6)</sup> and Balbiani<sup>(5)</sup>. The former gave an account of a spiral structure in chromosomes. (The arrangement of the chromomeres in a drawing by Sands<sup>(102)</sup>, who views the

structure as one of isolated chromatin bodies, is distinctly spiral.) The findings of Baranetzky have received support from a number of workers, notably Wilson<sup>(128)</sup>, Wenrich<sup>(126)</sup>, Fujii<sup>(47)</sup>, Kaufmann<sup>(61)</sup>, Sakamura<sup>(101)</sup>, Kuwada<sup>(65)</sup>, and Maeda<sup>(81)</sup>. Balbiani called attention to striations on the chromatic filaments of the nucleus. These striations were probably the result of a uniform distribution of chromatin bodies, the now recognised chromomeres, or possibly the spiral bands seen by Baranetzky.

Maeda<sup>(81)</sup> believes the chromosome spiral to be single. He illustrates such chromosomes in the microsporogenesis of *Lathyrus odoratus* in both the heterotypic and homotypic divisions. Kaufmann<sup>(61)</sup>, Sakamura<sup>(101)</sup> and Kuwada<sup>(65)</sup> regard the spirals as double. Favourable fixation indicates this. Kaufmann regards the chromatic substance as existing in the form of a pair of unbroken, intertwined spiral threads or "chromonemata." Martens<sup>(82)</sup> has interpreted chromosome structure on the basis of a discontinuous chromonema. Sands<sup>(102)</sup> also believes the chromatin to be grouped into bodies. On the basis of this latter point of view, the nuclear material can be regarded as a biphasic system of the emulsion type in which the chromatin is the dispersed phase (which is, however, unlikely) and the linin or achromatic substance the dispersion medium. Sands believes the linin to be in the form of an inner cylinder of jelly-like consistency in which the dispersed chromatin globules are imbedded. In this Sands upholds the older idea that chromatin and linin are distinct. That this may be true is beyond question, but it does not always appear from descriptions published that what is called linin is a different substance from the chromatin, as we shall later show.

Better known and more widely recognised is the "granular" structure of chromosomes. The "granules," or chromomeres, have been the subject of much speculation in the cytology of genetics. The chromosomes are regarded as linear aggregates of chromatin granules. The fact that these granules, the chromomeres, may exhibit a definite serial order has led to their being regarded as identical with those hypothetical "genes" in which lie, so it is presumed, the hereditary traits of organisms. The existence of chromosome granules has been brought into question by Grégoire<sup>(45)</sup> and others, though Chambers believes to have seen them as paired swellings in the diplotene stages of the living spermatocytes of grasshoppers examined *in vivo*.

There can be no doubt that many if not all of the granular hypotheses of chromosome structure are misinterpretations of an emulsion<sup>(12, 113)</sup>. Whether chromatin and linin are one and the same substance, namely, the dispersion medium of the emulsion (which seems more likely), or whether they are distinct substances, the chromomeres being the dispersed emulsion droplets (alveoli) and the linin thread being the continuous (interalveolar) medium, depends entirely on the picture which results on coagulation, and the observer's interpretation of it.

There does not appear to be any reason for regarding the dispersed globules in the chromosomal emulsion as the equivalents of the "genes" whose supposed linear arrangement has been the basis of genetical theories. Chamberlain<sup>(12)</sup> warns against such speculation, since he believes chromomeres on a linin thread to be a



misinterpretation of a "vacuolated" structure, and adds that "theories which cannot be reconciled with a vacuolated structure of the chromosome will have to be abandoned."

The visible emulsion is a superficial and secondary thing compared with that ultimate structure in protoplasm whose nature is not of an emulsion type. The elastic quality of chromosomes<sup>(16)</sup> has its basis in the specific structural character of the continuous phase of the emulsion, the chromosomal matrix. It is this matrix which represents the fundamental ground-substance. If we are to attribute peculiar vital properties to chromomeres they must then be of this fundamental ground-substance, in which case linin (as ordinarily described) and chromatin become identical. This is in harmony with the viewpoint that the chromomeres are the nodal points formed where the interalveolar layers meet at the junction of the emulsion globules.

Not all visible structures in protoplasm are necessarily to be interpreted on the basis of an emulsion. Chromosomes may contain structural features comparable to those in cells, such as fibres, mitochondria, etc. These need not be constituents of emulsions. The same may be true of the spirals found in chromosomes, though these may arise as a result of the fusing, at fixation, of the dispersed emulsion droplets, which, prior to fixation, had a spiral orientation.

*Chloroplasts.* Little is known of the structure of smaller protoplasmic inclusions. Most of them are too minute to reveal anything but the shape of the body as a whole. Chloroplasts, however, are large enough to show something of their internal anatomy. The most recent work on the structure of the chloroplast is that of Zirkle<sup>(133)</sup> who finds this body to be a hollow, flattened, prolate spheroid surrounding a central vacuole.

No information on the colloidal structure of chloroplasts or other small protoplasmic inclusions is available.

#### FIXATION AND ARTIFACTS.

Science advances through doubts, as well as through confident speculation. We are here for the moment interested in the doubts. Barely had cytological technique, with its methods of fixing and staining, got well under way than its results were questioned. Coagulated albumin does not look like the untreated material. What, therefore, can fixed and stained, dead and coagulated protoplasm tell us about the *living* substance? It was the cytologists<sup>(33)</sup> themselves who first doubted their own findings. The attack on the value of cytological work culminated in the experiments of Fischer<sup>(29)</sup> and Hardy<sup>(47)</sup>, who found that the visible structure seen in proteins after treatment with coagulating reagents varies with the kind of fixative used.

Fischer was able to produce fine and coarse granular and reticular structures of considerable variety by using solutions of peptone, haemoglobin, nuclein, serum albumin, paraglobulin, etc., and by precipitating these with fixing reagents such as osmic acid, chromic acid, Flemming's and Altmann's mixtures. The structural pictures obtained look very much like those in prepared tissues, and there is no



reason to believe that the particular type of structure seen exists in the protein before coagulation. It could not, since the same protein gives unlike pictures with different fixatives. Hardy, directing his attention especially against Bütschli's hypothesis of protoplasmic structure, arrived at the same conclusions as did Fischer. Before taking up Hardy's work, a word should be said about the extent to which the criticisms of cytological methods have gone in showing that all results based on fixed material are unreliable.

Our knowledge of cell anatomy would not have progressed nearly so rapidly without the help of cytological methods. The entire series of events which we collectively call mitosis were first revealed and thoroughly understood in fixed material. Even to-day, while many of these events have since been seen in the living cell, thus substantiating the results got by fixation, some of the finer details would not yet be known had not cytological methods brought them to view.

The case of spindle fibres illustrates the attitude of workers toward fixed material. Unlike chromosomes and mitochondria which can be seen in the living state (their presence in stained sections was also at first regarded as an artifact), spindle fibres have not been seen in normal living material. Strangeways and Canti<sup>(119)</sup> have described the gradual appearance of the spindle fibres on fixation of tissue-culture cells with chromo-acetic acid. The most careful scrutiny fails to reveal any sign of fibres in the spindle of untreated cells. Their appearance in fixed material is thought to be a post-mortem coagulation phenomenon. Fischer<sup>(29)</sup> believed he had proved this. Consequently, many have stated that the spindles do not exist as such in the normal cell.

There are two reasons for regarding the spindle-fibre formation of mitotic figures as typical of the living and normal dividing cell. First, Lewis<sup>(73)</sup> brought about their appearance in living cells by adding acid, and, on washing the acid away, the visible fibres disappeared, after which the cell continued division quite normally. The rendering visible of the fibres was, therefore, rightly regarded by Lewis<sup>(73)</sup> as a reversible coagulation. It is doubtful if an artifact is of the reversible type.

The second bit of evidence in favour of the permanent nature of spindle fibres as structures in dividing cells is the regularity of their occurrence in all cells in which indirect division is known to occur, and also their uniformity in appearance and location not only in all cells but with all fixatives.

McClung<sup>(88)</sup> has shown that in the first spermatocyte metaphase of Orthoptera killed with cyanide the spindle is long and clean, the chromosomes extended and well distributed, and the cytoplasm clear and bright, but when the insect is killed with xylol the spindle is short and restricted, the chromosomes are contracted and crowded, and the cytoplasm is granular and hazy. Such facts leave little doubt that the spindle is a pre-existing thing distorted by fixation methods but not an artifact. Bělař<sup>(8)</sup> has compared fixed material with the living, and finds the structures of the two identical in dividing snail spermatocytes.

Whether we regard the spindle fibres as threads, rows of granules, or lines of force, is of no matter. If fixation causes previously existing lines of force to appear

as fibres this is sufficient, for, after all, what are lines of force if not the linear orientation of particles, whether electrons or protoplasmic granules.

Much that is seen in fixed material is artifact, but much is a fairly good if not always an exact counterpart of that which exists in the living state.

Hardy's criticism of the worth of anatomical studies on coagulated protoplasm was directed primarily against the alveolar structure which Bütschli believed to exist not only where it is visible, but in all protoplasm. Its invisibility Bütschli thought due to insufficient optical difference between the two phases of the emulsion. He, therefore, maintained that fixation merely made visible a pre-existing alveolar structure. Hardy, on the contrary, thought that this structure was a post-mortem thing with no counterpart in the living material.

Bütschli<sup>(11)</sup> expressed his point of view in the following words: "Moreover, the fibrous alveolar structure, which is distinctly exhibited by these apparently homogeneous pseudopodia here and there after suitable fixation and coloration, is a sure proof that they do not lack the honeycombed structure, but only that as the result of special conditions it is no longer to be observed in life, nor even to a great extent after fixation." From Plateau's<sup>(12)</sup> calculations on soap bubbles it is possible to establish the fact that the thickness of the fluid lamella at the summit of a soap bubble may sink to 0.0001 mm. Hence, there is theoretically nothing to oppose the notion that the walls of the microscopic alveoli of protoplasm may, under certain circumstances, become thinned out to invisibility. To affect this a comparatively slight attenuation would be quite sufficient, since often when the alveolar walls are visible in living protoplasm they are already so delicate and faint that if attenuated to a relatively slight extent they would disappear from vision.

Hardy<sup>(13)</sup> was on the other hand equally convinced of his point of view. He says, "I have determined the existence of a solid framework having an open net structure in the following gels: in white of egg coagulated by corrosive sublimate, heat, potassium bichromate, or a trace of potassium sulpho-cyanate: in a hydrogel of silicic acid, and in gelatine coagulated by sublimate, ammonium, bichromate, or formalin: in a gel of celloidine produced by the action of chloroform or an ether-absolute solution, and lastly in common black india-rubber. In other words, and this is what I wish to insist upon here, the very essence of the process of fixation is the separation of solid from liquid and the formation thereby of a structure which may have had no counterpart whatever before fixation occurred."

To Bütschli's counter statement that one cannot question the existence of an alveolar structure in protoplasm because it is "frequently to be observed quite plainly in the living condition and therefore cannot be an artificially produced appearance of precipitation or coagulation," Hardy replies, "An examination of the original memoirs convinces me that it is very doubtful whether the structure in question has been observed in actually normal living cells."

This last statement of Hardy weakens his whole argument. Like Bütschli, he went too far. The one thought alveolar protoplasm to be of universal occurrence, and the other denied its existence entirely. It is always unwise emphatically to deny a thing in a field of investigation in which one has not worked. No proto-

zoologist could make such a statement as did Hardy, a statement which has been supported by others. Thus Gaidukov<sup>(42)</sup> says "the origin of the foam structure in protoplasm is a pathological or post-mortem process."

It is beyond all doubt that certain protoplasmic regions exhibit a beautiful alveolar structure in the living and normal state.

Mast<sup>(84)</sup> has brought interesting support to Bütschli's contention that where the alveolar structure is not visible in the living and unstained condition, it is nevertheless there. Mast has observed Brownian movement in the firm outer protoplasmic layer ("plasmagel") of amoeba. Since Brownian movement is incompatible with very high viscosity, Mast concludes that the protoplasm must contain minute and invisible vacuoles in which the particles move. This is further supported by the restricted range of movement of the particles which do not progress beyond the boundaries of a very small area.

Since the ectoplasm is of "gel" consistency, the only possible deduction is "that the plasma gel contains a rigid framework with numerous spaces filled with a substance which has the properties of a fluid in which the granules are suspended." "It would thus appear that in amoeba we have in the plasma gel a typical Bütschlian alveolar structure" (84).

Hardy's work is of great value and has done much to make workers cautious in interpreting what they see in fixed material, yet no cytologist who has approached the subject seriously and thoroughly doubts the existence of alveolar protoplasm. We do doubt, on the other hand, that this structure is a fundamental one. Wilson<sup>(131)</sup> expresses the consensus of cytological opinion when he says that "the visible alveoli or emulsion-like structure are neither a primary nor a universal characteristic of protoplasm."

#### COLLOIDAL INTERPRETATIONS OF PROTOPLASMIC STRUCTURE.

*The emulsion hypothesis.* The micellar hypothesis of the structure of protoplasm and of non-living gels advanced by Nägeli<sup>(91)</sup> antedates Bütschli's<sup>(11)</sup> alveolar hypothesis by eight years, but since the former leads more directly to modern theories of gel structure, while the hypothesis of Bütschli, stimulating as it was, has led to some fundamental misconceptions regarding the structure of the living and of non-living jellies, it is convenient to consider the alveolar hypothesis first.

Bütschli regarded protoplasm as possessing the structure of a foam except that the contents of the scattered globules is liquid and not gaseous. This foam-like structure he called "alveolar," the globules being alveoli ("little chambers").

By "foam" Bütschli meant "emulsion." He rather naively defends his use of the term "foam" by citing so eminent a physicist as Quincke, who, "without hesitation," calls systems "foams" even "when they are composed of two fluids." It matters little what we term a system so long as we know what is intended. Since a liquid-liquid system is meant here and since a colloidal system of this type is technically known as an emulsion—a foam being a system in which gas is dispersed in a liquid—then "emulsion" is the proper expression to use.

The emulsion theory of protoplasmic and of gel structure in general (Bütschli

included non-living systems in his theory as well as the living one) received serious consideration from the colloidal chemists. The theory was, however, early discarded by them partly because of the large size of the alveoli ( $1-2\mu$ ) and partly because it failed to meet requirements other than those of size of unit, which, in the colloidal world, must be below  $0.1\mu$ . (This last statement, while true in the strict and limited sense, is not rigidly adhered to, especially when emulsions are the colloidal systems under consideration.) Its utter inadequacy as a structural basis for elastic jellies is a much more fundamental reason than size for the discarding of the alveolus as the unit of gel structure.

The belief that hydrophilic colloidal systems (those capable of imbibition) are fine emulsions, led to the classification of colloids into suspensoids and "emulsoids" (93). This terminology and the conceptions on which it is based were not accepted by many leading colloid chemists and are now regarded as fallacious by practically all workers in the field (35). Hatschek (50), from a convincing mathematical analysis, concludes that "the theory that gels consist of two liquid phases must be pronounced untenable." Ellis (24) had previously shown that fine neutral emulsions are model suspensoids and not hydrophiles. Duclaux (22) regards liquid and solid suspensions as having nothing to do with what he calls the "true colloids," *i.e.* the gels and jellies.

Biologists (15, 115) on the other hand have, in the main, clung to the belief that protoplasm is, in its ultimate fundamental structure, an emulsion.

The most ardent supporter of the "emulsoid" (emulsion) hypothesis of the structure of protoplasm is Fischer (30), who says that the characteristics of lyophilic colloidal systems are best explained on the assumption that they are systems of mutually soluble phases and are capable of forming two types of solutions, the one of protoplasm (or of protein or phenol) in water and the other of water in protoplasm (or in protein or phenol).

Clowes (18) has based a very ingenious explanation of cell permeability on an emulsion structure of the protoplasmic membrane.

Belief in the emulsion structure of protoplasm has led to many incompatible statements. We read that protoplasm is a "gel," but if we accept the word of practically all colloid chemists we must realise that emulsions do not form "gels." We read further that "phases may be reversed in such a hydrogel." Phase reversal is a property of emulsions and not of gels. Laing and McBain (66) have shown that the electrical conductivity of soap gels is identical with that of the sol, thus precluding phase reversal. Spek (116) says that it is quite improbable that the dispersed phase in protoplasm could become the dispersion medium.

In its microscopically visible structure protoplasm is, beyond doubt, in many instances an emulsion. Possible, also, is it that the minute ultramicroscopic particles which are seen dancing, scintillating in great numbers against a dark background when protoplasm is viewed with indirect illumination, are liquid in nature. If this is true, then the ultramicroscopic living solution is an emulsion. But back of this lies the fundamental structure which is the ultimate seat of vital processes, as it is also the mechanical basis of such physical properties of



protoplasm as elasticity, coagulation, and imbibition. This structure is not an emulsion.

A crude comparison may be made between protoplasm and milk.

Milk consists of an emulsion of butter-fat dispersed in an aqueous medium of casein and other substances. Naturally, if the fat droplets in milk are removed then we no longer have milk, but if we are interested in some other property of milk such as coagulation, and are in search of the ultimate structure which is the seat of this phenomenon, then we must look beyond the visible emulsion. It is the casein in milk which coagulates. The fat globules play no active part in this process.

Coagulation phenomena play an important rôle in cell processes. Usually, coagulation is regarded as incompatible with life but not necessarily always. Heilbrunn<sup>(53)</sup> believes that the mitotic spindle arises as a direct result of coagulative changes. Viscosity changes during egg cleavage are discussed by Chambers<sup>(13, 15)</sup>.

It is impossible to find in an emulsion (of pure oil and water) a mechanical explanation of such physical properties as coagulation, imbibition, and elasticity.

Chodat<sup>(17)</sup> points out that it is the meshes of a fibrous, not an emulsion, structure of protoplasm which hold the water of imbibition.

A comparison of the microscopic structures of elastic and inelastic soaps<sup>(109)</sup> argues against an emulsion structure for elastic jellies of which protoplasm is one. Elastic soaps contain long, slender, crystalline fibres, while the microscopic particles seen in an inelastic soap are irregularly spherical, resembling chalk-dust.

Among chemists, the conclusion is all but unanimous that jellies are not fine emulsions. Among biologists, opinion is divided in regard to protoplasm. If we accept the chemical viewpoint and further grant that protoplasm is a hydrophilic colloid—and on this practically all biologists are agreed—then we cannot escape the conclusion that protoplasm in its ultimate structure is not an emulsion.

Bütschli's hypothesis and all others based on emulsions must go if they pretend to account for the primary structure of protoplasm.

*The micellar hypothesis.* The botanist Nägeli<sup>(91)</sup> is given credit for advancing the first concrete theory of the structure of protoplasm and of colloidal gels. He regarded both as built up of molecular aggregates which he termed "micellae." These "micellae" have been erroneously regarded as synonymous with the hypothetical "gemmules" of Darwin, or the "physiological units" of Spencer; at least from the chemical viewpoint such a comparison is fallacious. It is in the chemical sense that the term "micellae" is used here.

Whether micellae, in the sense of molecular aggregates, are actually the structural units of jellies, and if they are, what their precise nature is, are both much debated questions in colloidal chemistry. We can, however, definitely state that micellae cannot rightly be regarded as liquid droplets and therefore that Nägeli's hypothesis remains quite distinct from that of Bütschli's. It is, consequently, inconsistent to support both an emulsion and a micellar hypothesis of protoplasmic structure.

There can be no doubt but that structural units of colloidal dimensions are to



be found in protoplasm and in certain non-living gels, but it appears that they are merely coarser manifestations of the finer structure which lies beyond. Their presence, however, throws important light on the nature of the finer structure.

Ettisch and Szegvari<sup>(26)</sup> find that dark-field illumination reveals rod-shaped micellae in the fibrous strands of frog connective tissue.

Combes<sup>(20)</sup> interprets the adsorption of water by the cytoplasmic hydrogel on the basis of the behaviour of colloidal micellae.

Scarth<sup>(104)</sup> believes chloroplasts to be built of colloidal micellae which are of the nature of fluid crystals, that is, a predominant number of the molecules are oriented and the micellae themselves have a more or less regular orientation.

Frey<sup>(38)</sup>, from the double refraction of submicroscopic particles, concludes that distinct cellulose micellae exist in the cell wall. This last reference is to material that is not protoplasmic, but the relation between protoplasmic structure and that of all non-living gels and jellies is very close. Frey believes to have proven Nägeli's theory that the cell wall is built up of oblong optically anisotropic, crystalline micellae.

Bachmann<sup>(4)</sup> has described the coming together of ultramicroscopic masses of gelatine to form the structure of the cold hydrated gel. He thinks that these visible micellae are preceded by, and in turn built up of, smaller aggregates, amicrons and submicrons, whose ultimate structural unit is the molecule.

McBain<sup>(87)</sup> regards soap gels as of micellar structure.

Gortner<sup>(43)</sup> believes that gelatine-aggregates or micellae become more and more interlaced at increasing concentrations of gelatine. He indicates that micellae and colloidal fibres, or even linear molecules, may become identical. No one knows just what a micella is, anyway, and it is quite possible that there are as many different kinds of micellae as there are gels.

Assuming that the structure of proteins and of protoplasm is a micellar one, in the original Nägeli sense, *i.e.* an open one, with relatively large structural units, then surface phenomena such as adsorption must take place within the jelly and play a prominent rôle in the behaviour of all lyophilic colloidal systems.

Freundlich<sup>(36)</sup> calls attention to an interesting case of the very gradual but complete chemical change in a tissue by adsorption. The chitin plate of crustacean chelae is developed from sinew. The plate, although quite different from sinew in construction, has, throughout, the structure of the sinew from which it evolved. Similar changes, the first step of which is an adsorption process, are found in the non-living organic and inorganic world. Zeolite undergoes such a change, and the transformation of cellulose into nitrocellulose is another instance. Freundlich<sup>(36)</sup> has given the name "permutoid" to these substances, and emphasises the possibility of clearly distinguishing true chemical reactions from adsorption phenomena in the permutoids.

The anti-micellar viewpoint is held by Procter<sup>(99)</sup>, Wilson<sup>(132)</sup> and Loeb<sup>(80)</sup>. (Procter is somewhat more lenient in his attitude toward the much abused micella.)

Loeb minimises the importance of micellae in colloidal, and therefore in protoplasmic, behaviour. He does not regard such a unit as existing in hydrated blocks

of gelatine. They are formed as localised aggregates when concentrated hot gelatine is cooling but are not to be found in the gelatinised jelly. The micellae of gelatine are, therefore, only the precursors of a gel and do not exist as such in a continuous gel. A block of gelatine is a huge micella; the two, the micella and the gelatine block, differ only as to size, and the ultimate structural unit of both is the same, namely, the molecule. It is this unit which Loeb (80) and his predecessors Procter (99) and Wilson (132) regard as the structural unit of jellies. This leads us to a consideration of the molecular hypothesis of protoplasmic structure.

#### MOLECULAR HYPOTHESIS OF PROTOPLASMIC STRUCTURE.

The province of colloidal chemistry has been much disputed since Graham founded this special branch of science. The term "colloid" is discarded by some and "colloidal state" substituted. But even this substitution, while a move toward clarity in classification, does not help us very much. Duclaux (22) has taken a step in another direction by adhering strictly to the etymological meaning of the word "colloid." He excludes all systems which are not "glue-like."

The customary definition of the colloidal state is that in which the dispersed particles are above the molecule in size and below microscopic visibility ( $0.1\mu$ ), or as one scientist puts it (87), that state of matter which recognises the micella as its unit of structure. Should it turn out to be that hydrated gelatine is built up of molecules and not of micellae, then the above definition would exclude so typical a colloid as gelatine. It is not our purpose here to attempt to settle this much controverted matter, but simply to point to the confusion that exists and to the doubt in men's minds as to the precise nature of the structural unit of a colloidal system of the protein type.

No one questions that gelatine at temperatures of  $36^{\circ}\text{C}$ . and over is a molecular dispersion with a tendency to hydrolyse to simpler molecules such as gelatoses. At lower temperatures the molecules aggregate into groups, or micellae. What happens at the gelatinising point no one knows. Wilson (132) and Loeb (80) believe that the structural unit of a hydrated block of gelatine is the molecule. This is also the opinion of Langmuir (67), who has estimated the size of the hydrated gelatine unit and finds it to be of the order of atomic and not colloidal dimensions.

Procter (99) was among the first to point to the strictly chemical (*i.e.* stoichiometric) behaviour of protein systems in contrast to the purely colloidal theories of Freundlich, yet Procter does not seem adverse to admitting the possibility of the molecular fibres aggregating into larger groups of linear micellae. He does, however, regard the structural unit of a jelly as of molecular dimensions in so far as it is sufficiently fine to keep the whole within the range of molecular forces. There is some doubt as to the size, but less as to the shape of the unit of elastic jellies. It is linear; if a molecule, then a long slender one of the amino-acid type; if a colloidal particle, then a tenuous crystalline fibre. It is the interlocking of these linear units which gives to jellies their rigidity and high degree of elasticity.

A remarkably sudden collapse of the firm structure formed by the interlocked

fibrous units may occur both in protoplasm and in non-living gels. Pressure may cause the instantaneous breakdown of the mitotic figure of a dividing echinoderm egg (108). Mechanical disturbance may likewise bring about the sudden liquefaction of gels, for example of iron oxide (105).

That the structure of jellies and of protoplasm is an entanglement of linear units, a "brush heap," is strongly suggested by their elastic properties.

The elasticity of protoplasm is easily demonstrated by stretching living cytoplasm, the protoplasmic membrane or nuclei, between microneedles (111).

The elastic properties of very dilute colloidal solutions may be ascertained by placing a minute ( $15\mu$ ) nickel particle within the solution with the aid of micrurgical technique (37, 96) and attracting the particle magnetically. The same method has been applied to protoplasm. The particle is held in suspension by the "rigidity" of the thin solution. It does not travel at a uniform rate when attracted by the magnet, indicating heterogeneity in the gel structure. When the magnetic force is taken away the particle retraces its path, thus demonstrating elastic qualities (37). Glycerine, with a viscosity value of 830, is barely capable of holding a  $10\mu$  metal particle in suspension (within the solution). If the particle is magnetically attracted while it slowly falls, it shows no tendency to return on release of the magnetic force. A soap solution of but twice the viscosity of water may yet possess sufficient "rigidity" to hold a  $10\mu$  metal particle and exhibit high elastic qualities. The rigidity and elasticity are due to the interlocking of structural units of a fibrous nature. This has been shown to be true for soap solutions (109). Inelastic solutions possess no such structure but are built up of spherical particles.

The consistency of protoplasm has received much more attention than has its elasticity. Viscosity values throw some light on the nature of protoplasmic structure; thus high viscosity indicates the gel state among lyophilic colloidal systems. Weber (127) calls attention to the rôle of viscosity in determining the form of protoplasts, and Lloyd (78) has shown the importance of viscosity changes in conjugation in *Spirogyra*. Heilbrunn (53) and Chambers (14, 15) have associated viscosity changes with mitosis. Significant as viscosity is in the study of protoplasm, it is the elastic and rigid properties which so clearly indicate its jelly nature and suggest its structural design.

The linear structural units of elastic jellies are apparently crystalline in nature. Procter (99) was one of the first to suggest this.

Scherrer (106) and Sponsler (118) have determined the crystalline character of such elastic organic matter as starch, cellulose, gelatine, silk, ramie, brain, nerve, and muscle. The method used is that of von Laue and Bragg (9), wherein the space lattice formed by the atoms of the crystal serves as a diffraction grating for Röntgen rays which produce symmetrical diagrams on a photographic negative.

Scarth (103), from observations on the *Spirogyra* chloroplast through crossed nicols (*i.e.* a polarising prism below the object and another perpendicular to it in the microscope eyepiece), concludes that protoplasm is similar to a liquid crystal. He makes the further interesting observation that, after a chloroplast has become liquid under the influence of reagents which lower its viscosity, and has again

"gelated," the double refraction returns with even greater brilliancy, showing that the molecules or crystalline particles again orientate along definite axes.

Muscle and contractive tissues generally are double refracting. Mathews (86) states that muscle as a whole is more comparable to a liquid crystal than to a gel.

The substance which bathes the structural framework of the linear crystalline units cannot be other than a complex aqueous solution of salts, carbohydrates, organic food matter and waste material. Lepeschkin (70) emphasises the fact that the dispersion medium of protoplasm is not water but an organic liquid consisting of loose chemical combinations of proteins and lipoids in which water is soluble to a limited extent.

The concept of an interlacing mass of amino-acid chains or slender crystalline fibres makes it possible to interpret the mechanism of some of the physiological properties of protoplasm, which cannot be done, or at least has not been done, on the basis of any other type of system. One such property is the immiscibility of protoplasm in water.

There has long waged a controversy over the miscibility or immiscibility of protoplasm in water. The contention of those who support the miscibility side of the controversy is that the living substance is kept from mixing in its surrounding aqueous medium by the presence of an outer oily layer. Protoplasm, when cut or torn with microneedles, immediately forms a new surface over the wounded part (54, 107), but it is not this surface alone which prevents loss of the inner mass by diffusion into the aqueous medium. The protoplasm resists disintegration because of its own peculiar structure.

Isolated bits of protoplasm frequently "struggle" to maintain their identity. A severed droplet of protoplasm may not completely round up, part of its surface remaining ragged and exposed. At such times there is often no indication of miscibility at the still exposed region until a complete breakdown in the entire mass occurs, when the protoplasm quickly diffuses into the water or coagulates. This sudden disintegration of a protoplast due to a complete collapse in structure may take place with the rapidity of an explosion. Protozoa and eggs of echinoderms frequently literally blow up. That same structure which makes it possible for a protoplast to maintain its identity in water is also responsible for the taking in of water by protoplasm. This is an imbibition process and is not to be confused with miscibility in the sense of solubility (17, 107).

Closely associated with the property of elasticity is that of glutinosity. Lewis (75) states that cells are held together by adhesion. Kredel (63) supports this in finding that embryonic tissues are devoid of intercellular bridges. It is the stickiness of cells which keeps animals from falling apart, and things stick because their surfaces become entangled.

An interesting feature of this problem is the coexistence in a system of the properties of elasticity (and rigidity) and the capacity flow. Protoplasm possesses both of the seproperties; it is elastic (and rigid), yet flows. Investigators have been inclined to emphasise that property of protoplasm which best supports their conception of the mechanism of protoplasmic behaviour.



Bayliss (7) repeatedly emphasises the fluid nature of protoplasm. The earlier cytologists questioned those speculations which attributed to protoplasm a framework on the ground that the living substance is liquid and flows. Others (110) maintain that the living substance is, in all its properties, essentially a jelly.

Emphasis on the liquid nature of protoplasm is hardly compatible with the high tensile strength of the living substance which was ingeniously determined by Pfeffer (97), to whom is due the credit of making the first actual measurement of a physical property of protoplasm. He tied minute weights to the end of a freely hanging strand of the plasmodium of the slime mould *Chondrioderma*. He ascertained the maximum load which the protoplasmic thread would support, and calculated the tensile strength or, as he expressed it, the cohesive force, of the protoplasm. This was found to be 50 mg. per sq. mm. ( $\frac{3}{4}$  mg. actual weight on a strand measuring 0.3 mm. in diameter). A pull of 50 mg. causes no stretching. The maximum load which a protoplasmic strand would support, but with gradual stretching in time, was 210 mg. per sq. mm.

We get tensile strength in cloth and rope by making them of threads.

Scarth (104) points out that streaming protoplasmic threads exhibit elasticity.

Szegvari (122) has attempted to visualise the mechanism involved in a system whose structure is such as to give elastic qualities and yet permit flowing.

An interpretation of the structural features of liquids, especially those which can be stretched, is of great significance and involves all our present-day knowledge of the polarity, orientation, and shifting of molecules. The problem is a very large one and would carry us far afield if we were to attempt to review the work done, but a few remarks on the fundamental principles involved will help to visualise a mechanism which will permit liquid jellies, such as thin gelatine, or protoplasm, to have elastic qualities and continuity in structure, and yet possess the capacity to flow. We have only to think of a framework in which the points of contact of the structural units are not rigidly fixed, but hold as do the poles of two magnets, firmly yet moveably. Two linear molecules may be in contact and cling fairly tightly to each other, yet permit sliding or turning at the point of adherence. The chemist has the same sort of thing to deal with in the phenomenon known as tautomerism.

Tautomerism has to do with the shift of an atom from one position to another and does not involve any question as to whether, in so shifting, the atom is to be regarded as a free atom. The mobility of a liquid is thus due to a shifting of the relative positions of atoms. It is such linkages between one molecule and another which give elastic qualities to liquids whose molecules are of a suitable shape (linear) and suitably orientated.

These theories are as applicable to protoplasm as to any non-living system. One must grant that life is, so to speak, a new departure, but we can never hope to understand it if we do not attempt to apply known simple chemical and physical laws.

Linear molecules properly orientated form the structural background which accounts for the phenomenon of polarity, so universal in living things. The



interlocking of these orientated linear molecules gives to protoplasm its continuity in structure without which life is inconceivable.

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# EVOLUTIONARY SEQUENCE AND AFFINITIES AMONG PROTOPHYTA<sup>1</sup>

By F. E. FRITSCH.

(Received November 20, 1928.)

(With Seven Text-figures.)

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## INTRODUCTION.

THE designation Protophyta may aptly be applied to all simple uni- and multi-cellular organisms that obtain their nourishment after the manner of a plant, although a rigid definition is impossible and undesirable. They include all the elementary types of holophytic plant-life of which we have any knowledge and afford the only available data on which to base our views as to the nature of the earliest vegetable organisms and the various stages that were passed through in the gradual elaboration of a plant-body. They thus afford material for unlimited phylogenetic speculation, and the majority of treatises on Algae published during the last thirty years abound in phylogenetic trees, all of which are highly hypothetical and many of which are shown to be incorrect within a few years of their publication. Very many facts of fundamental importance from the evolutionary standpoint can, however, be gleaned from a survey of the simpler Protophyta, without indulging in undue speculation, and it is with this aspect of the subject that the present article more especially deals.

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The modern era in the study of Protophyta began, towards the end of the last century, with the establishment of the concept of the origin of the different classes of Algae from a flagellate ancestry, a doctrine too well known to require a detailed elucidation here. The ground had been prepared by gradual recognition of the fact that the Chlorophyceae included two distinct types of green Algae, the one truly green and storing starch, the other yellow-green and storing oil. The first important step in this direction was taken by Borzi (1895, p. 199) who established a group Confervales including unicellular, colonial, and filamentous types (*Ophiocytium*, *Mischococcus*, *Conferva*<sup>1</sup>, etc.) having these and other characters in common; A. Braun (1855, p. 49) had long previously drawn attention to their affinity. In 1897 Bohlin (1897), in a critical investigation of *Conferva*<sup>1</sup> and *Ophiocytium*, further emphasised the close relationship of the forms included in Borzi's Confervales. In the same year he (Bohlin, 1897 a) described a motile unicellular Flagellate, exhibiting the essential characteristics of the Confervales, under the name of *Chloramoeba*, while in 1899 Luther established the genus *Chlorosaccus* for a palmelloid form with the same peculiarities. Recognising that, apart from other resemblances, there was a close correspondence between *Chloramoeba* and the zoospores of *Chlorosaccus* and *Conferva*<sup>1</sup> he (1899, p. 17) advocated the removal of the whole set of forms from the Chlorophyceae and their incorporation in a distinct class, Heterokontae, with an ancestry among Flagellates of the *Chloramoeba*-type. In the following year F. F. Blackman (1900), in an article dealing with the relation of Algae and Flagellata, drew the attention of English botanists to the new viewpoint, while in 1901 Bohlin once more reviewed the whole situation. The first detailed classification of green Algae, in which these modern views find expression, is that of Blackman and Tansley (1902), where the name Isokontae<sup>2</sup> was suggested for the large residue of the Chlorophyceae, the latter name disappearing altogether from the reprint published in the following year (Blackman and Tansley, 1903).

The two classes of green forms thus distinguished have been accepted by most subsequent authorities and are now recognised as so distinct that there is little question of any direct affinity between them. The true green Algae (Isokontae) are characterised by possessing large and often elaborate chloroplasts (commonly only one or two in the cell), containing the same pigments (chlorophyll *a* and *b*, carotin, xanthophyll) as those of higher plants and probably in about the same proportions; pyrenoids, usually surrounded by a starch-sheath, are very commonly present, and with few exceptions starch is stored after active photosynthesis. The cell-membranes consist largely of cellulose. The motile stages (motile unicells, zoospores,

<sup>1</sup> Now known as *Tribonema*.

<sup>2</sup> This name is preferable to that of Chlorophyceae, which stood both for Isokontae and Heterokontae, so that the use of the designation Chlorophyceae may easily be misleading. Printz (1927) retains the name Chlorophyceae with the subdivisions Euchlorophyceae (the true green forms), Conjugatae, Heterokontae, and Charophyta. This however implies an affinity between Heterokontae and the true green Algae for which there is no evidence at all and such a practice is to be deprecated. If the name Chlorophyceae be retained at all, it should be used solely for the pure green Algae, called Isokontae in the present article.

gametes) possess two (Fig. 1 *A*) or four (Fig. 1 *C*) equal cilia<sup>1</sup> (sometimes other numbers) arising at the anterior end. The yellow-green Algae (Heterokontae) usually have in their cells a number of discoid chloroplasts containing an excess of xanthophyll and always devoid of pyrenoids; starch is never stored, while oil (fat) accumulates after active photosynthesis. The cell-membranes are generally rich in pectic substances and not uncommonly consist of two overlapping pieces. The motile stages possess one long and one very short cilium (the latter sometimes lacking?) arising at the anterior end (Fig. 2 *A*, *K*).

It will be realised that the distinctive characters of the two classes (like those of the other classes of Protophyta to be mentioned later) are essentially physiological, depending on the pigmentation of the plastids and the types of metabolism associated with them, as evidenced by the substances stored after photosynthesis and by the chemical nature of the cell-membranes. That the diverse classes are also in general characterised by other features (*e.g.* number and arrangement of cilia, special characters of the reproductive organs) shows that the physiological differences are fundamental and go hand in hand with other distinctive features.

Bohlin (1901, p. 25) applied the designation Stephanokontae to *Oedogonium* and its allies, implying a distinct origin for this group from a flagellate stock having the crown of cilia of the well-known zoospore, although he included his Stephanokontae as a subdivision of Chlorophyceae. Blackman and Tansley (1902, p. 20), however, raised them to the rank of a separate class, and this procedure has been followed by many subsequent authors. Blackman and Tansley also (*loc. cit.*) ranked the Conjugatae as a distinct class of green Algae, bestowing upon them the name Akontae (Blackman and Tansley, 1903, p. 2), thus following the practice initiated by Wille (1897) and adopted by most later authorities. A separation of either Conjugatae or Oedogoniales from the rest of the green Algae (Isokontae) must, however, obscure the essential principles underlying the present-day concept of algal evolution, since in the pigmentation of their chloroplasts, in the possession of pyrenoids with a "starch-sheath," in the storage of starch, and the chemical nature of their cell-walls these two groups are altogether Isokontan. Nor do they stand more isolated from the bulk of the Isokontae than do many other recognised families of this class (*e.g.* Coleochaetaceae, Vaucheriaceae). There can be little doubt that they belong to the same evolutionary series as the remainder of the true green Algae (*cf.* also below, p. 111).

#### 1. ISOKONTAE (CHLOROPHYCEAE).

Despite the removal of the genera now classed in Heterokontae, there still remains within the Isokontae a very large number of diverse forms, representing practically every conceivable type of simple plant-body, occupying an immense variety of habitats, and exhibiting a very extensive range in reproductive methods. There is in fact no other class of simple organisms showing anything like as wide a scope in all these respects. A brief review of this extensive class may be

<sup>1</sup> The term "cilium," which is in general use in botanical terminology, is used throughout for the motile organs of the flagellate types. There is no fundamental difference between the "flagella" of these forms and the cilia of an algal zoospore.

undertaken at the outset and will serve to indicate the main directions in which Protophyte evolution has taken place.

The motile type is represented by a wide diversity of unicellular and colonial forms, the majority of which, in contrast to other classes, are provided with a firm cell-membrane and exhibit sexual reproduction. A small number, usually grouped as Polyblepharidaceae<sup>1</sup>, are stated to have naked protoplasts, and these include a little known colonial type (*Raciborskiella* Wislouch, 1924) and a number of colourless forms (*Polytomella* Aragao, 1910; *Furcilla* Stokes, Fig. 1 K, cf. Pascher, 1927, p. 113). It is, however, not certain that all the Polyblepharidaceae are really devoid of a membrane. This family no doubt constitutes an artificial assemblage, some forms perhaps being primitively naked, whilst others have secondarily lost their membrane. Thus, *Dunaliella* (Fig. 1 G, Teodoresco, 1905 and 1906), which is often placed in this family, is probably to be regarded as a reduced member of Chlamydomonadaceae (Pascher, 1912, p. 283), whilst Skuja (1927), in describing a stigma-bearing form of *Furcilla lobosa* showing traces of a reduced chloroplast, advocates a position for this genus among Chlamydomonadaceae in the neighbourhood of *Brachiomonas* (cf. also Printz, 1927, p. 446). The majority of the Polyblepharidaceae certainly appear specialised rather than simple (cf. for instance, *Pyramimonas*, Fig. 1 C), and the old view that regarded them as the most primitive known members of Isokontae might with advantage be abandoned. Sexual reproduction is known in *Dunaliella*, *Pyramimonas* (Printz, 1927, p. 43), and *Phyllocardium* (Korschikoff, 1927).

Apart from these few naked forms, the motile type among Isokontae appears in two well-marked series, the Chlamydomonadaceae and Sphaerellaceae, represented by *Chlamydomonas* and *Sphaerella* (*Haematococcus*<sup>2</sup>) respectively, and each including colonial types<sup>3</sup>. In the former there is typically a basin-shaped chloroplast, often with a single median pyrenoid, and there are usually two contractile vacuoles near the anterior extremity (Fig. 1 A); in the latter there is a diffuse ill-defined peripheral chloroplast with two or more pyrenoids, the contractile vacuoles are scattered, and the protoplast is provided with branched processes traversing the thick mucilaginous wall (Fig. 1 B). The relation of these two series to one another is not clear, but the Sphaerellaceae are probably derived forms. Only few members of this family are at present known, *Sphaerella*, the colonial *Stephanosphaera*, and probably most forms of *Volvox* (Crow, 1918; Shaw, 1922, p. 225; Pascher, 1927, p. 404); it does not appear that any of the sedentary Isokontae are related to this type.

On the other hand the Chlamydomonadaceae are represented by an abundance of unicellular and colonial types, the former including the huge genera *Chlamydomonas* and *Carteria*. The sixteen unicellular genera distinguished by Pascher (1927,

<sup>1</sup> The number of cilia in this family is very varied, e.g. 6-8 in *Polyblepharis*, 5 in *Chloraster*, 4 in *Pyramimonas* (Fig. 1 C), 3 in *Trichloris* (Fig. 1 J; Pascher, 1927, p. 103), 2 or 4 in *Spermatozopsis* (Korschikoff, 1913), 2 in *Asteromonas* (Artari, 1913), and one in *Monomastix* (Scherffel, 1912).

<sup>2</sup> *Sphaerella* has priority over *Haematococcus*.

<sup>3</sup> It does not appear advisable to separate the unicellular and colonial forms, as Printz (1927) and Pascher (1927) do.

p. 136) are connected with one another by all kinds of transitions, so that a sharp delimitation is impossible (Pascher, 1927, p. 136, footnote). It seems that here,

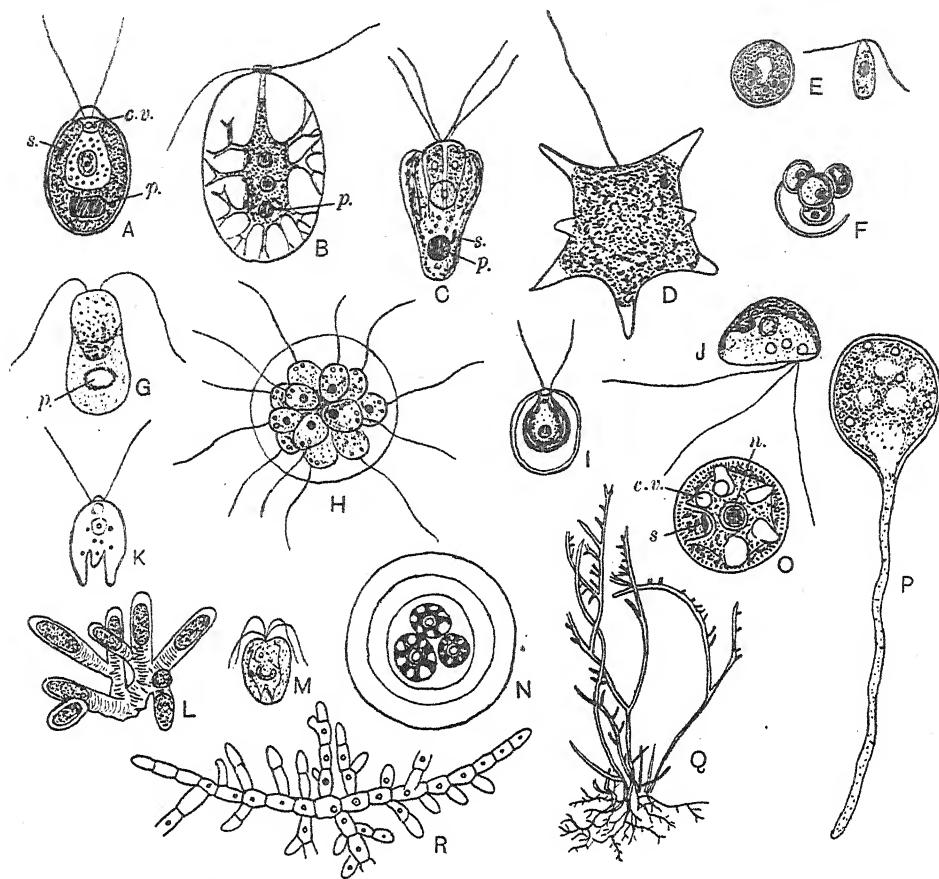


Fig. 1. Types of Isokontae (Chlorophyceae). A, *Chlamydomonas angulosa* Dill (after Dill). B, *Sphaerella buetschlii* (Blochmann) (after Blochmann). C, *Pyramimonas tetrarhynchus* Schmarda (after Dill). D, *Chloroceras corniferum* Schiller (after Schiller). E, *Chlorococcum humicolum* (Naeg.) Rabenh.; on the right a swarmer (after Bristol). F, *Chlorella vulgaris* Beij., showing liberation of daughter-cells (after Grintzesco). G, *Dunaliella salina* Teodoresco (after Teodoresco). H, *Mastigospheera gobii* Schewiak. (after Schewiakoff). I, *Coccomonas orbicularis* Stein (after Stein). J, *Trichloris paradoxa* Scherffel and Pascher (after Pascher). K, *Furcilla trifurca* Pascher (after Pascher). L, *Prasinocladus lubricus* Kuck., dendroid colony (after Kuckuck); M, swarmer of same. N, O, *Asterococcus superbus* (Cienk.) Scherffel (after Scherffel); N, colony; O, single cell. P, *Protosiphon botryoides* (Kütz.) Klebs (after Klebs). Q, *Stigeoclonium tenue* Kütz., heterotrichous filament (after Huber). R, *S. lubricum* Kütz., creeping basal system (after Berthold). c.v. contractile vacuoles; p. pyrenoid; n. nucleus; s. stigma.

as in other groups of Isokontae, practically every conceivable variant of the central type has been realised. The majority of the members are bi- or quadri-ciliate (*Carteria*, the colonial *Spondylomorom*<sup>1</sup>), but there are also uniciliate forms (*Chloro-*

<sup>1</sup> Closely related forms are however biciliate, e.g. *Chlamydotryps* (Korschikoff, 1924) which Printz (1927, p. 61) includes under *Uva* (Playfair, 1914, p. 108).

*ceras* Schiller, 1927, Fig. 1 D; *Mastigosphaera* Schewiakoff, 1893, Fig. 1 H). The colonial members, the bulk of which are biciliate, do not exhibit the same extreme diversity as the unicellular forms, and include the very instructive series *Gonium-Pandorina-Eudorina*, etc. The colonial Chlamydomonadaceae show their high specialisation, not only in the oogamy of the more advanced types, but also in the definite integration of the colony (coenobium), the full number of constituent individuals being determined in the embryo-stage, so that cell-division only occurs during the production of new coenobia. *Volvox* itself is almost certainly heterogeneous, representing the culmination of two or more evolutionary series within this group, but whether a generic separation on the lines advocated by Shaw (1919, 1922, 1922 a and b, 1923) is feasible seems doubtful (cf. Pascher, 1927, p. 462). It would appear that within the confines of *Volvox* there are forms with the *Chlamydomonas*-type of cell-structure, as well as others (apparently in the majority) which show that of a *Sphaerella*. In particular, in *Volvox mononae* (Smith, 1920) protoplasmic connections seem to be altogether lacking at all stages of the life-history and the cells have a basin-shaped chloroplast with a single median pyrenoid. It is probable that in the future some forms of *Volvox* will be relegated to Sphaerellaceae, others to Chlamydomonadaceae, and this serves to emphasise the fact that the coenobial Volvocales cannot be considered apart from the unicellular members of the same series.

A number of unicellular Volvocales, grouped in the artificial family Phacotaceae, have their cells enclosed in a special thick, often offstanding, envelope provided with one or two anterior apertures for the protrusion of the two cilia. Here belong *Coccomonas* (Fig. 1 I), *Dysmorphococcus* (Takeda, 1916), *Phacotus*, and *Pteromonas*, the last two with a bivalved envelope; it is probable that in some of these forms the actual individual is naked. This *encapsuled* habit is a variant met with also in other classes of Protophyta.

There remain a series of colourless forms which, though often grouped as Polytomaceae for convenience, should no doubt be distributed among the other families of Volvocales (Printz, 1927, p. 64). *Polytoma* (Fig. 6 A) is closely related to *Chlamydomonas*, the quadriciliate *Tetrahlepharis* (Senn, 1897) to *Carteria*, while *Chlamydothlepharis* (Francé, 1894, Fig. 6 C) is obviously a saprophytic member of Phacotaceae<sup>1</sup>. Here, therefore, as in other classes (cf. p. 135), colourless saprophytic derivatives have arisen at different levels from the holophytic series.

All the Isokontae so far considered (the Chlamydomonadales) are motile throughout the greater part of their life, though many cease movement at the time of reproduction. Two sets of sedentary colonial forms are plainly derivable from such free-moving unicells, and, in view of the ease with which they revert to the motile phase, are evidently more closely related to it than the remaining sedentary Isokontae. A considerable number of the forms included here retain eye-spots and contractile vacuoles during the motionless phase (e.g. *Schizochlamys*, Scherffel, 1908, p. 787; *Asterococcus*, Fig. 1 O, Scherffel, 1908 a, p. 767). In the one case the

<sup>1</sup> Van Tieghem's (1880) *Sycamina* is regarded by some as a colourless colonial member of Volvocales, but this is open to considerable doubt.



colonies are *dendroid* (Chlorodendrales) and are well illustrated by *Chlorangium* and *Prasinocladus* (Fig. 1 L; Zimmermann, 1924; Kuckuck, 1894; = *Euglenopsis* Davis, 1894; *Chlorodendron* Senn, 1900), the former with 2-, the latter with 4-ciliate swarming stages (Fig. 1 M). The two genera do not appear closely related, and the same applies to the lesser known forms of this series, such as *Ecballiocystis* (Bohlin, 1897 b, p. 7; Fritsch, 1918, p. 494). Many regard Borzi's *Physocytium* as a simple member of this group.

In the other series the colonies are *palmelloid* (Tetrasporales) and composed of numerous cells embedded in mucilage. It can hardly be doubted that such forms have arisen from the well-known tendency of many Chlamydomonadaceae to form *Palmella*-stages, in fact in one family (Palmellaceae, with *Asterococcus*, Fig. 1 N, *O Gloeocystis*, *Palmella*, etc.) the habit is so reminiscent of such stages that they still afford scope for speculation as to polymorphism. The other family (Tetrasporaceae) is more sharply characterised by its pseudocilia, the groups of cells in fours, and the often definite shape of the mucilage-masses. Its direct affinities are obscure.

The three series Chlamydomonadales, Chlorodendrales, and Tetrasporales comprise the motile members and their immediate derivatives, and may well be collected in the one group Volvocales. Contrasted with this we have the Chlorococcales<sup>1</sup>, a group of essentially sedentary unicellular and colonial forms producing swimmers only as a passing phase in connection with reproduction or dispensing with motility altogether. This *chlorococcoid* type is a far more definite step in the direction of the typical plant than is realised in the dendroid or palmelloid colony. Its origin from motile forms is, however, readily visualised if the brief motionless phase that often precedes cell-division in the unicellular Chlamydomonadales be supposed to become indefinitely prolonged at the expense of the period of free movement. There results a motionless unicell like *Chlorococcum* (Fig. 1 E) whose contents periodically divide to form swimmers; sometimes, however, the latter fail to develop cilia and acquire a membrane already prior to their liberation (aplano-spores, Bristol, 1920), so that motility is altogether suppressed. This is the normal state of affairs in *Chlorella* (Fig. 1 F). A certain number of Chlorococcales produce swimmers like *Chlorococcum*, but a much larger number seem to lack them and to reproduce after the manner of a *Chlorella*.

Oltmanns (1904, p. 170) first employed this feature in the classification of the group, and it was subsequently fully elaborated by Brunnthaler (1913), who divided the Chlorococcales into Zoosporinae (zoosporic forms) and Autosporinae (azoo-sporic forms). Many subsequent writers have followed the same course, but, while in the present state of our knowledge it is perhaps a convenient systematic distinction, it no doubt obscures affinities (cf. Geitler, 1924). It is clear that all the azoo-sporic forms did not originate from a common stock. Complete suppression of motility no doubt occurred again and again in the evolution of the group, and the coenobial members (Hydrodictyaceae, Coelastraceae), for instance, are certainly more closely related than their relegation to distinct series would imply. *Sorastrum*,

<sup>1</sup> Usually known as Protococcales, but this name should be abandoned (cf. West & Fritsch, 1927, p. 95).

till recently classed in Coelastraceae, has now been shown to reproduce by swarmers after the manner of Hydrodictyaceae (Probst, 1916; Geitler, 1924), while *Pediastrum* may behave as an azoosporic form (West, 1916, p. 217); a reproduction of *Crucigenia* by zoospores has also been rendered probable (Chodat, 1925, p. 445). It is not unlikely that, with the present widespread discovery of new Chlamydomonadales, types may be found that will justify a closer approximation of certain genera of this group and of Chlorococcales. In other words there is no reason to assume that the origin of the green chlorococcoid types was monophyletic.

The *filamentous habit* is more highly developed and more widely represented among Isokontae than in any other class of Protophyta, if we leave the brown and red seaweeds out of consideration. It is possible to distinguish the five groups Ulotrichales, Chaetophorales, Oedogoniales, Conjugatae, and Siphonales (West & Fritsch, 1927, p. 59), which will here be dealt with only from the evolutionary aspects.

Filamentous types no doubt originated direct from motile unicells, the view that assumed a derivation from palmelloid types with a filamentous tendency being altogether unsupported by fact. The mode of origin of the filament is illustrated by the behaviour of the zoospore in any filamentous Alga. The phylogeny of the filament is recapitulated by its ontogeny! The evolution of the filament and of all other types of multicellular plant-bodies resulted from the acquisition of the faculty of limitless division of a purely vegetative type. In Volvocales and Chlorococcales there is no vegetative division, cell-division being always closely linked up with reproduction and involving a rejuvenescence of the protoplast with casting off of the parent cell-membrance at an earlier or later state (Fig. 1 F). In vegetative division, on the other hand, such as characterises the filamentous types, a dividing cell is partitioned by a septum which, in the majority of Isokontae, arises as an annular ingrowth from the longitudinal walls and, subsequent to nuclear division, gradually cuts across the protoplast (West and Fritsch, 1927, pp. 28, 142; Printz, 1927, p. 7). The two cells thus produced adopt the membrane of the parent-cell, except for the intervening septum which is new and common to both (cf. however Pascher, 1924, p. 152). A consequence of this method of cell-increase is a tendency for the products to cohere, unless subsequent splitting of the septum occurs, as in *Stichococcus* and *Pleurococcus* for instance. One may look upon the filament also from the mechanical point of view, the septa giving mechanical stability to a lengthening cylinder, such as might be obtained by the elongation in one direction of a *Chlamydomonas*-cell. Vegetative division among unicellular types is confined to *Pleurococcus* and a few others (Printz, 1927, p. 99), the former being now commonly regarded as a reduced member of Chaetophorales (Chodat, 1894; Oltmanns, 1922, p. 304).

The ordinary filamentous type among Isokontae is represented by the Ulotrichales and Chaetophorales, the other three groups being specialised in various directions. In many Ulotrichales the filament is a simple unbranched thread of uniform cells, usually attached by a more or less elaborate basal cell, although there are some branched types (cf. below, p. 112) and two interesting sets of forms

(Ulvaceae, Prasiolaceae) in which division takes place in several planes with the development of parenchymatous foliaceous expanses; these, however, lack the morphological differentiation attained by some of the foliaceous seaweeds. The central types (*Stigeoclonium*, Fig. 1 Q, R, *Trentepohlia*) among Chaetophorales (Fritsch, 1916) possess a plant-body differentiated into a prostrate system of creeping threads serving *inter alia* for attachment to the substratum and a more or less richly branched projecting system. This type is represented in three distinct families of Chaetophorales—the Chaetophoraceae, Trentepohliaceae, and Coleochaetaceae; it is, moreover, met with in other classes of Protophyta and may be described as the *heterotrichous type* (Fig. 1 Q). There is much variation in Chaetophorales in the relative differentiation of the prostrate and projecting systems, and reduction of the latter has led to a whole series of specialised prostrate or discoid types (*Aphanochaete*, *Protoderma*, *Phycopeltis*, etc.), while among Chaetophoraceae we have in *Draparnaldia* the most highly elaborated form (with a simple reproduction however) known in Isokontae. It is not unlikely that some of the Ulotrichales and Chaetophorales may have arisen from a common stock, although the filamentous Isokontae are almost certainly polyphyletic.

The swimmers in these two groups are partly bi- and partly quadri-ciliate (Pascher, 1907), which may imply a derivation from distinct series of motile Isokontan unicells; in the same way the Oedogoniales may have arisen from unicellular forms with many cilia, although such are unknown. It is, however, equally possible that the multiciliate habit of the swimmer of Oedogoniales is a secondary acquisition. The Oedogoniales are highly specialised, as evidenced by the complex cell-division and the advanced oogamy with the differentiation of "dwarf-males," and no simpler forms are known which might link the group to other filamentous or to unicellular types.

To some extent this is also true of the Conjugatae, although one of their essential characteristics, the peculiar process of sexual reproduction known as conjugation, can be related to the sexual fusion found in some species of *Chlamydomonas* (e.g. *C. braunii*) in which the gametes are provided with cell-walls (Blackman & Tansley, 1902, p. 168). The Conjugatae include a series of unbranched filamentous types (Zygnemales) in which there is a clear advance from isogamy to anisogamy, and a series of unicellular forms, the Desmids, which exemplify in a striking manner the potentialities for the varied evolution of a single-celled organism. No Conjugate produces swimmers, and it is within the realm of possibility that the group arose from a stock that never possessed motility, although the numerous azoosporic Chlorococcales, which are almost certainly derived from zoosporic forms (cf. above), make such an interpretation doubtful.

The chief characteristic of the Siphonales is the rare production of septa, the plants being coenocytic with numerous nuclei and small discoid chloroplasts, commonly without pyrenoids, embedded in the parietal cytoplasm (the *siphonous habit*). The group is poorly represented in freshwaters, but has attained to a very varied and often complex development in the sea, especially in warmer climates. To deal with these elaborate Siphonales, which in part develop their

thallus on lines repeated among the brown and red seaweeds, is outside the scope of this article. The origin of the group is perhaps to be sought in forms like *Protosiphon* (Fig. 1 P; Klebs, 1896, p. 187)<sup>1</sup>, referred by some to Chlorococcales (Oltmanns, 1922, p. 261; West and Fritsch, 1927, p. 111), by others to Siphonales (Blackman and Tansley, 1902, p. 115), a sufficient indication of its intermediate character. Many of the zoosporic Chlorococcales (*Chlorochytrium*, etc.), as a matter of fact, have cells which are multinucleate at maturity. The Siphonales are best regarded as a group in which elongation is unaccompanied by the customary septation, so that the structure is mechanically unsound, and other methods for attaining stability have had to be evolved (cellulose-bars in *Caulerpa*, rope-like twining of the threads in Codiaceae, etc.). In many members of the group septa are only produced to delimit the reproductive organs (sporangia, gametangia), but in a certain number (*Valonia*, *Siphonocladus*) rather numerous septa appear in the older plant. It has been customary to regard the Cladophoraceae and Sphaeropleaceae as related to these Siphonales with septate tendencies and to class all such forms as Siphonocladeae by contrast to the aseptate Siphoneae.

This attitude, however, places undue stress on a single character, the coenocytic structure. There can be little doubt that Cladophoraceae and Sphaeropleaceae are not nearly related to the true Siphonales, in fact they show more evident affinity with the Ulotrichales (West and Fritsch, 1927, p. 148; Fritsch, 1929). The chloroplasts in the two families are fundamentally of the same type and evidently related to those of Ulotrichales. The cells of *Rhizoclonium* (Peterschilka, 1924) are commonly uninucleate. Both families lack the specialised sporangia and gametangia of Siphonales, the quadriciliate swarmer of Cladophoraceae is unknown in the latter group, and in *Sphaeroplea* reduction would appear to occur in the zygote as in Ulotrichales, the filament being haploid. Schussnig (1928) has recently described the occurrence of reduction in the gametangia of *Cladophora glomerata*, and similar conclusions were communicated by Higgins at the Glasgow meeting of the British Association for an unnamed species of this genus. These observations, implying that the *Cladophora*-filament is diploid, constitute a resemblance to Siphonales (cf. Williams, 1925), the significance of which cannot at present be estimated.

It is possible that, even with the elimination of these two families, the Siphonales are still heterogeneous. What, for instance, is the affinity of the common freshwater and terrestrial genus *Vaucheria*? In its advanced oogamy, its peculiar zoospore, and the production of oil as a photosynthetic product it stands far apart from all other coenocytic green Algae. Owing to its metabolism it has been referred to Heterokontae, but so much speaks against the reference that no recent authorities have accepted this view. *Dichotomosiphon* (Ernst, 1902), in habit and reproduction very similar to *Vaucheria*, produces starch, and that has militated more than anything else against the idea of a Heterokontan affinity. It should be realised, too, that the synzoospore of *Vaucheria* is Isokontan in type and that the spermatozoids, although aberrant, are in no way like the swarmers of Heterokontae. There is in

<sup>1</sup> Cf. also *Urnerella* (Playfair, 1918, p. 529), *Sphaerosiphon* (Schussnig, 1915), and *Follicularia* (Miller, 1923) which Printz (1927, p. 151) classes together with *Protosiphon* as Protosiphonaceae.



fact very little to lend support to the view of an Heterokontan affinity, although *Vaucheria* and *Dichotomosiphon* may not of course be as closely related as their resemblance implies. It has been suggested that these Vaucheriaceae may "represent an algal group parallel with the Phycomycetes and possibly having a common origin with them" (West and Fritsch, 1927, p. 293), a matter that will be referred to again below. *Vaucheria* stands as isolated as do nearly all the oogamous members of Isokontae.

One may picture the evolution of the Isokontae as starting with a unicellular ancestry, the exact type of which is apparently no longer represented at the present day, but which was almost certainly motile and probably very similar to a *Chlamydomonas*. From this ancestry there arose numerous distinct evolutionary lines leading to the development of manifold types of body, each in its turn with numerous variants. Thus, starting with the motile holophytic unicell, we have the motile colourless unicell, the encapsuled unicell, the motile colony, the dendroid colony, the palmelloid colony, the chlorococcoid unicell and colony (zoosporic and azoosporic), the simple filament, the branched filament, the heterotrichous filament, the prostrate epiphytic type (incl. discoid forms), and the siphonous type. It is unlikely that many of these principal categories have ever originated directly from one another (except branched from unbranched filaments, prostrate types from heterotrichous filaments). They would appear to represent as many different attempts at the building of a body from the unicellular ancestry, and one of the most significant results of recent investigation of Protophyta is the recognition of the fact that the same types of construction have been evolved separately time and again from the unicellular ancestry in other classes. This parallelism in evolution will be clearly recognised in the subsequent considerations (cf. also Pascher, 1914; Fritsch, 1927). The relation of the different types of body to the motile unicell is probably, in all classes, the same as has been outlined for Isokontae in the preceding pages.

## 2. HETEROKONTAE.

When first segregated from the Isokontae this class included only relatively few forms, but during the present century many additional members have been discovered, and it has become increasingly apparent that there exists a far-going correspondence between the two classes, to which Pascher (1913, p. 6) first drew attention. The Heterokontae do not, however, exhibit anything approaching the multiplicity of forms to be seen among Isokontae. Few examples of the motile unicell are known, such as *Chloramoeba* and *Heterochloris* (Fig. 2 A-C; Pascher, 1925, p. 23), all being naked and of rare occurrence. *Heterochloris* may at times assume a completely rhizopodial form<sup>1</sup> (Pascher, 1917 a, p. 19) (cf. below, p. 139). The motile colony and the encapsuled type are both unrepresented, nor are any permanently colourless members of the Chloramoebales known, although *Chloramoeba* itself develops a colourless habit if supplied with organic nutriment in darkness

<sup>1</sup> *Stipitococcus* (West and West, 1898, p. 336), of which a second species has been described by Schmidle (1902, p. 151), is a unicellular epiphyte provided with a stalked envelope and bearing, in the case of Schmidle's species, an apical rhizopodium (Pascher, 1925, p. 27). Its affinities are very uncertain and either or both species may belong to another class.



(Bohlin, 1897 a, p. 515). The dendroid type is represented by *Mischococcus* (Fig. 2 F), and a number of palmelloid forms (Heterocapsales) exist, such as *Chlorosaccus* and the very common plankton Alga *Botryococcus* (Carlson, 1906, p. 141).

The chlorococcoid type (Heterococcales) is rather abundantly represented and

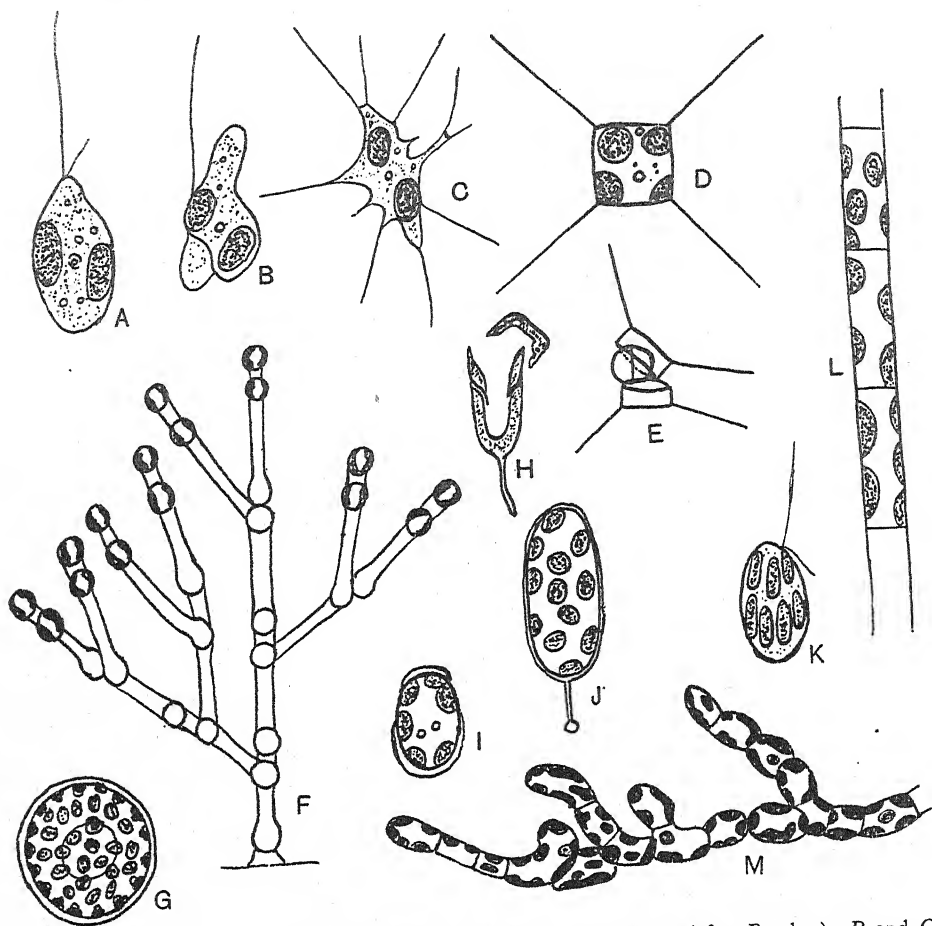


Fig. 2. Types of Heterokontae. A-C, *Heterochloris mutabilis* Pascher (after Pascher); B and C show transitions to a rhizopodial condition. D, E, *Pseudotetraëdron neglectum* Pascher (after Pascher); D, single cell seen from the surface; E, liberation of cyst. F, *Mischococcus confervicola* Naeg., dendroid colony (after A. Braun). G, *Botrydiopsis arhiza* Borzi (after Borzi). H, *Ophiocytium*, showing structure of wall (after Bohlin). I, *Tribonema bombycinum* Derb. et Sol., cyst (after Klebs). J, *Characiopsis teres* Pascher (after Pascher). K, *Tribonema bombycinum*, zoospore (after Luther). L, *Tribonema vulgare* Pascher (after Pascher). M, *Monocilia viridis* Gerneck (after Gerneck).

in part the relevant forms are so similar to members of Chlorococcales (Isokontae) that, prior to the clear recognition of the differences between the two classes, they were placed in the same genus. Thus, many species of the epiphytic *Characiopsis* (Fig. 2 J) were first included in *Characium*, the type-species of *Chlorobotrys* (Bohlin, 1901 a, p. 34) was first described as a *Chlorococcum*, *Botrydiopsis* (Fig. 2 G)

has a considerable superficial resemblance to a small *Eremosphaera*, while the Isokontan genus *Tetraëdron* is paralleled by the Heterokontan *Pseudotetraëdron* (Fig. 2 *D, E*; Pascher, 1913, p. 1). The two common oceanic plankton Algae, *Halosphaera* and *Meringosphaera*, are also members of the Heterococcales (Pascher, 1915, 1917). The group includes both zoosporic and azoosporic forms.

The filamentous Heterokontae are few and exhibit little specialisation. The very widely distributed freshwater genus *Tribonema* (Fig. 2 *L*) has unbranched threads without any differentiation among the cells, whilst the branched type is seen in *Monocilia*<sup>1</sup> (Fig. 2 *M*; Gerneck, 1907, p. 263) which is as yet only known from cultures, so that its natural condition is uncertain. The only representative of the siphonous type is the common mud-Alga *Botrydium*, now clearly established as a member of Heterokontae (Kolkwitz, 1926, p. 539); more elaborate forms have been described from India (Iyengar, 1925).

The scanty occurrence of filamentous and siphonous forms (*i.e.* of all types of more elaborate body) implies a far less vigorous development than obtains in the Isokontae, and this is in accordance with the fact that only a few of the more specialised members of Heterokontae exhibit sexual reproduction and that this has not passed beyond the level of isogamy (Scherffel, 1901, p. 149). A peculiar and widespread feature of the class, which does not however appear to occur in all the genera, is the fact that the cell-walls are composed of two overlapping pieces (*cf.* Fig. 2 *E, H*). This is found also in a few Isokontae (*Microspora*, Desmids) and is of course a characteristic of the Diatoms (Bacillariales); in the latter case it possibly implies some relationship (*cf.* below). Many Heterokontae form resting-spores with a wall composed of two equal or unequal pieces (Fig. 2 *I*; Pascher, 1921, p. 242).

The few ciliated members of Heterokontae that are at present known without exception show what have been regarded as "flagellate" characters (*cf.* Blackman, 1900, p. 667; Blackman and Tansley, 1902, p. 22), *i.e.* they are devoid of a cell-wall, their periplast is more or less rigid but usually admits of some change of shape, multiplication is effected by longitudinal division, the protoplast readily encysts, and sexual reproduction is not known to occur. Some of the palmelloid members (*e.g.* *Chlorosaccus*), possibly all, also show these features. The many motile and palmelloid Isokontan types are, on the other hand, "algal" in organisation, being provided with a firm cell-wall and generally exhibiting sexuality. In view of the obvious parallelism between the two classes, however, this distinction between algal and flagellate forms appears of little consequence (West and Fritsch, 1927, p. 19), and it will be realised that "algal" characters have been assumed at an earlier stage in the evolution of the one than in that of the other. These conclusions would be of little interest if they applied only to the two classes Isokontae and Heterokontae, but it is evident that an analogous evolutionary sequence has been followed in *all* classes of pigmented Protophyta, although the features associated with "algal organisation" have appeared, if at all, at different points in the sequence in the different classes. There is reason to believe that every class of holophytic

<sup>1</sup> This is synonymous with the *Heterococcus* of Chodat (1913, p. 177).

Flagellates could potentially have acquired algal characters, although on the present evidence some have failed to do so.

### 3. CHRYSOPHYCEAE.

The preceding remarks could not be better illustrated than by a consideration of the Chrysophyceae (Pascher, 1914, p. 143; Pascher, 1925 *a*), our knowledge of which, as that of other flagellate groups, has been very considerably widened in the last two decades, thanks mainly to the magnificent work of Pascher. Until relatively recent times the Chrysophyceae were only known to include a wealth of flagellate types, the Chrysomonadales, whose members on the whole favour pure waters and seem often to attain a maximum abundance in cold weather or in the cold streams and pools of mountainous tracts. In the sea they are represented by the Coccolithophoridae and other forms, and they also seem to play a conspicuous part in certain kinds of salt-marsh (Conrad, 1926).

The class is distinguished by the golden yellow (sometimes brown) colour of the chromatophores which are most usually few (often one or two) in number; the colour is due to varying amounts of accessory pigments (Gaidukov, 1900, p. 331) whose exact nature is at present little known. Pyrenoid-like bodies have been recorded in a number of cases (Doefflein, 1923, p. 267), but are rare. Starch is not produced, although Pascher (1912 *a*, pp. 180, 194) records starch-like grains in a few cases; the products of photosynthesis are, however, mostly stored as oil and as colourless, highly refractive, usually rounded lumps of *leucosin*<sup>1</sup> whose chemical composition is unknown (Fig. 3, *A, K, N, I*). A very characteristic feature of the class are endogenously formed, silicified cysts<sup>2</sup>, first fully described by Scherffel (1911, p. 334; cf. also Pascher, 1925 *a*, p. 491). The protoplast secretes near its periphery a hollow silicified membrane (Fig. 3 *D, E*) provided with a small aperture towards one side; the outer surface may subsequently become elaborately sculptured. Finally the external protoplasm passes through the pore into the interior of the cyst and the aperture is closed from the inside by the production of a special silicified plug, usually more or less conical in shape (Fig. 3 *F*).

The motile type is better represented in this class than in any other group of pigmented Protophyta, but there is greater diversity in ciliary characters than is usually found, and this appears to extend also to the reproductive cells of the advanced sedentary types. Three distinct series of the flagellate Chrysomonadales have long been known, all with apical attachment of the cilia, viz. the Chromulinales with a single cilium (Fig. 3 *A*), the Ochromonadales with one long and one short one (Fig. 3 *B*), and the Hymenomonadales (Pascher's Isochrysidales) with two equal cilia (Fig. 3 *J*). Recently Conrad (1926, p. 219) has described *Prymnesium* (Fig. 3 *C*), with one short and two long cilia, the only example of this type of ciliation at present known in the class. Several species of *Chromulina* and *Ochromonas* approach one another very closely (Oltmanns, 1922, p. 21), except for their ciliary characters, and there is probably a near relationship between some of the members

<sup>1</sup> According to Pascher (1921, p. 247) leucosin is also found in Heterokontae.

<sup>2</sup> Also found in diatomaceous earths (cf. Henderson, 1925, pp. 136, 142).

of the three series. Holophytic nutrition is the rule, but the holozoic method is occasionally resorted to (cf. Conrad, 1926, p. 174).

No purpose would be served by a consideration of the diverse variants of the ordinary motile unicell within the class and we may turn at once to notice the many special developments. The encapsuled type is widely represented and is seen in its most typical form in *Chrysococcus* (Fig. 3 G) among the Chromulinales. Here the envelope, which is separated by a slight space from the contained individual, is continuous, more or less spherical, often brown-coloured owing to impregnation with iron salts, and sometimes silicified (Conrad, 1926, p. 176). Several authorities (Pascher, 1912 a, p. 193; Conrad, 1926, p. 176) have suggested a more or less close relation between such types and the Silicoflagellata (Dictyochidae), which have been studied by Borgert (1891) and Lemmermann (1901).

The majority of the encapsuled Chrysophyceae are, however, epiphytes in which the individual is located within a wide offstanding envelope of diverse shape and provided with a broad aperture through which the cilia protrude (Fig. 3 H). Direct motility is only resorted to at times of reproduction and, except at such periods, the protruding cilia probably serve in the main to waft solid food-particles to the contained individuals. In some cases indeed (*Chrysopyxis bipes*, cf. Pascher, 1912 a, p. 163) the cilium is modified in the sedentary individual to form a branched rhizopodium (cf. also p. 139). This encapsuled epiphytic type is represented in all three series of Chrysomonadales by large genera with numerous species, viz. *Chrysopyxis*, *Derepyxis* (Fig. 3 H), and *Dinobryon* (sect. *Epipyxis*).

A number of the more advanced forms exhibit special developments of the periplast which, as Pascher (1912 a, pp. 157, 187) has shown, usually go hand in hand with a more elaborate system of contractile vacuoles and more complex nuclear structure. Among Chromulinales this is seen in the Mallomonadaceae. The relatively large individuals of *Mallomonas* have many small imbricating silicified scales deposited in the periplast, some or all of the scales bearing delicate, hinged, likewise silicified needles which are commonly of considerable length and no doubt aid in flotation. In the recently described *Conradiella* (Pascher, 1925 b, p. 566; Conrad, 1926, p. 188) the envelope takes the form of annular silicified plates which are presumed to have arisen by the fusion of separate scales like those of *Mallomonas*.

Among the biciliate Hymenomonadales these more complex types are represented by the marine Coccolithophoridae (*Pontosphaera* (Fig. 3 I), *Syracosphaera*, etc.), where the individuals are enveloped in a layer of mucilage within which are deposited a large number of calcareous rings or discs (coccoliths) of diverse shape (Lohman, 1902; Schiller, 1925 and 1926). New coccoliths are successively formed internal to the old ones which gradually drop off. They are common objects in marine deposits, past and present. Conrad (1915, 1926, p. 196) has shown that *Hymenomonas*, one species of which (*H. roseola* Stein, Fig. 3 J) is occasionally found in fresh water, belongs to this family and has described a further form (*Coccochrysis*) from a Belgian salt-marsh (1926, p. 199), whilst the *Pontosphaera sphagnicola* of Chodat and Rosillo (1925) constitutes another freshwater member of the family.



It is of interest that this essentially marine group has also got its freshwater representatives. Schiller (1926, p. 337) has shown that the marine genera *Rhabdosphaera* and *Discosphaera* are always devoid of cilia. Cysts have not yet been observed in this family (Schiller, 1925, p. 53).

The motile colony has been evolved in all three series of Chrysomonadales, being represented by *Chrysosphaerella* with one, *Syncrypta*, *Synura*, and *Chlorodesmus* with two equal, and *Uroglena* with two unequal cilia; several of these are not uncommon in freshwater plankton. *Chrysobotrys* (Fig. 3 K; Conrad, 1926, p. 216), belonging to the Ochromonadales, is of interest because the individuals are aggregated as in the Isokontan genus *Spondylomorum* (Volvocales). Many of these colonial forms show analogous complexities of the periplast to those above considered, and in *Skadovskiiella* (Korschikoff, 1927 a) there is an envelope formed by many minute siliceous rings with rod-like appendages, suggestive of the calcareous coccoliths of Coccolithophoridae. A description of the various colonial forms is unnecessary, but it may be noticed that the spherical type of colony predominates and that there is a less marked individuality than in the Volvocales, inasmuch as movement is irregular without a definite anterior end, the number of constituent individuals is a variable quantity, and multiplication is effected most commonly by fission of the mature colonies. Several interesting examples of incipient colony-formation have been recorded in *Chromulina* and *Ochromonas* (Pascher, 1910, p. 339).

The dendroid colony is exemplified by *Chrysodendron* (Pascher, 1927 b; Fig. 3 L), with cells having the structure of an *Ochromonas* and, in conformity with the dominance of the flagellate habit among Chrysophyceae, retaining the two unequal cilia even in the sedentary phase. According to Pascher the individuals very readily become detached from the stalks bearing them and, like so many other members of this class (cf. below), exhibit a great tendency to protrude pseudopodia in relation to animal nutrition. Conrad (1926, p. 180) has described similar dendroid colonies in *Pedinella hexacostata* (Chromulinales). The colonial members of *Dinobryon* and *Hyalobryon* are likewise dendroid in habit, but this is here combined with the differentiation of wide envelopes around the individuals and, in the case of the former, with a free-floating planktonic habit.

It will be clear that the motile individual in Chrysophyceae exhibits development in the most varied directions and, in view of the many discoveries of new types belonging to this class during the present century, it seems probable that by degrees nearly every conceivable variant will become known. The Chrysomonadales thus vie with the Volvocales in their many-sided evolution, but there is one marked point of contrast, namely, that in the former all the organisms involved possess "flagellate" organisation and that sexual reproduction is practically lacking. Schiller (1926, p. 336) has recorded the copulation of naked swimmers in *Dinobryon* and suspects a similar process in the Coccolithophoridae (*loc. cit.* p. 334), but both conclusions still await confirmation.

*Palmella*-stages are not uncommon in species of *Chromulina*, etc. (Pascher, 1912 a, p. 190), and in *C. mucicola* Lauterborn ciliated cells embedded in diffuent



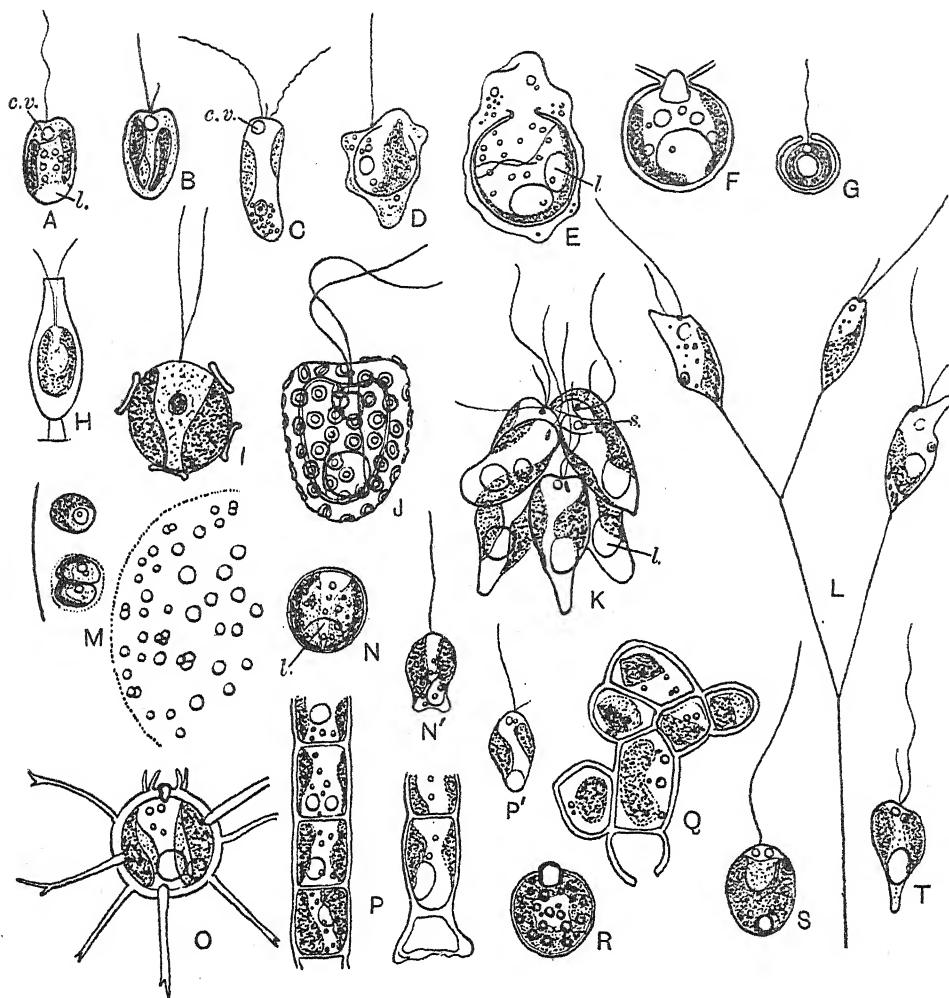


Fig. 3. Types of Chrysophyceae. A, *Chromulina ovalis* Klebs (after Klebs). B, *Ochromonas nutabilis* Klebs (after Pascher). C, *Prymnesium saltans* Massart (after Conrad). D-F, formation of cysts (after Pascher). G, *Chrysococcus rufescens* Klebs, encapsulated unicell (after Klebs). H, *Derepyxis amphora* Stokes, encapsulated epiphyte (after Stokes). I, *Pontosphaera huxleyi* Lohm. (after Schiller). J, *Hymenomonas roseola* Stein (after Conrad). K, *Chrysobotrys spondylomorum* Conrad (after Conrad). L, *Chrysodendron ramosum* Pascher, dendroid colony (after Pascher). M, *Chrysocapsa planktonica* Pascher, palmelloid colony (after Pascher); on the right small part of colony, on the left a few cells enlarged. N, *Chrysosphaera nitens* Pascher, chlorococcoid type (after Pascher); N', swarmer of same. O, *Echinochrysis chodati* Conrad (after Conrad). P, *Nematochrysis sessilis* Pascher, simple filament (after Pascher); on the right the basal part of a filament with attaching cell; P', swarmer of same. Q-S, *Thallochrysis pascheri* Conrad (after Conrad); Q, branched thread; R, cyst; S, swarmer. T, swarmer of *Echinochrysis chodati* Conrad (after Conrad). c.v. contractile vacuole; l. leucosin; s. stigma.

mucilage constitute the normal vegetative condition, swarming occurring only in connection with reproduction. Such stages point the way to the palmelloid Chrysocapsales (Pascher, 1925 *a*, p. 548), of which only a few are known, *e.g.* *Chrysocapsa* (Fig. 3 *M*), *Phaeosphaera* (West and West, 1903), *Pascherella* (Conrad, 1926, p. 221), *Phaeogloea* (Chodat, 1922, p. 87), and the remarkable *Hydrurus* which in its striking division of labour and great specialisation exhibits a far higher differentiation than any other known palmelloid type. Its distinctive swimmers, quite apart from other peculiarities, remove it from the remaining Chrysophyceae, and its immediate affinities are not at all clear.

The Chrysophyceae parallel to the Chlorococcales are the Chrysosphaerales (Pascher, 1925 *a*, p. 533), forms possessing algal organisation with firm cell-walls of unknown composition. The first genus to be described was *Chrysosphaera* (Pascher, 1914, p. 159, and 1925 *a*, p. 533), with cells lying isolated or in little clusters and containing a pair of parietal yellowish-brown chromatophores (Fig. 3 *N*). Reproduction is effected by division of the protoplast and by swimmers of the *Chromulina*-type (Fig. 3 *N'*). *Epichrysis*<sup>1</sup>, with similar structure and reproduction, is an epiphyte. None of the Chrysosphaerales form well-defined colonies such as are found in the Chlorococcales. Chodat (1922, p. 81) has applied the name Chrysostomataceae to a series of motionless unicellular freshwater forms, closely resembling the siliceous cysts produced by members of this class. They may represent a separate line of Chrysophyceae in which (as in some Dinophyceae) such resting-stages have become the normal vegetative condition, but their status is not yet clear (cf. Chodat, 1922, p. 82; 1925 *a*, p. 33; Pascher, 1925 *a*, p. 547) and many will probably prove to be the cysts of other members of the class. Conrad (1926, p. 222) has, however, described a genus, *Echinochrysis* (Fig. 3 *O*), which reproduces by zoospores of the *Ochromonas*-type (Fig. 3 *T*) and which appears to establish the independent existence of such forms.

Perhaps one of the most striking contributions to our knowledge of this class in recent years has been the description of a number of filamentous forms, grouped as Chrysotrichales (Pascher, 1925 *a*), among which the genus *Phaeothamnion* (Lagerheim, 1884) appears at length to have found a secure resting-place. The Chrysotrichales include unbranched filamentous types (the marine *Nematochrysis*, Fig. 3 *P*; Pascher, 1925 *a*, p. 511; Conrad, 1926, p. 224), branched filamentous forms (*Phaeothamnion*, *Chrysoclonium*, Pascher, 1925 *a*, p. 509, etc.), and prostrate branched or discoid types. The last are represented by *Thallochrysis* (Fig. 3 *Q*; Conrad, 1920, p. 180; 1926, p. 226) found in brackish water and forming more or less parenchymatous expanses, attached or free-floating, and by *Phaeodermatium*<sup>2</sup> (Hansgirg, 1892, p. 207; Pascher, 1925 *a*, p. 517) forming compact discs (up to 5 mm. in diameter) on the stones in cold streams. In all, reproduction by zoospores (Fig. 3 *P'*, *S*) with the habit of *Chromulina* or *Ochromonas* has been recorded and for most the characteristic siliceous cysts (Fig. 3 *R*) are known. Although it is not to be doubted that further filamentous forms will come to light, it already appears

<sup>1</sup> This is the same as *Phaeocapsa paludosa* Korschikoff (1924 *a*), according to Pascher.

<sup>2</sup> Cf. also *Phaeoplaca* (Chodat, 1925 *a*, p. 32), which is at present imperfectly known.

probable that, as in the Heterokontae, the filamentous Chrysophyceae will exhibit no marked vegetative differentiation, while their reproduction is of the simplest possible kind. Sexual reproduction is not known among them, since that recorded by Borzi (1892, p. 454) for *Phaeothamnion* is probably erroneous. The Chrysophyceae thus agree with the Heterokontae in the fact that the bulk of their development centres around the unicell and colony and in the relative simplicity of their reproductive processes, but the Chrysophyceae as a whole exhibit much greater diversity than the Heterokontae. The siphonous type has, however, so far not been observed in the former class.

#### 4. BACILLARIALES (DIATOMS).

The Bacillariales are a highly specialised group, any detailed consideration of which is altogether outside the scope of this article. Their affinities are obscure and they have been at various times regarded as allied to the Conjugatae, to the Phaeophyceae, and to the Peridinieae. None of these views have stood the test of a critical consideration, and it remains to be seen whether the most recent suggestion of Pascher (1914, p. 145; 1921) as to the existence of an affinity between Heterokontae, Chrysophyceae, and Bacillariales will be more successful.

Pascher's view is based on a number of similarities, viz. preponderance in all three classes of yellow or brown pigments in the chromatophores, the associated absence of starch and occurrence of oil as one of the usual assimilatory products, and certain resemblances in the structure of the cellular envelopes. These may well indicate a physiological relationship of some significance. Deposition of silica in the membrane is a feature of all three classes (mainly in the cysts in Heterokontae and Chrysophyceae), and the bivalved structure of the cell-wall typical of the ordinary Diatom-individual is seen both in the vegetative cells and the resting-stages of various Heterokontae (Fig. 2 *E, I*), while the cysts of Chrysophyceae (Fig. 3 *F, R*) likewise always have an envelope composed of two pieces. The endogenous cysts, formed by the marine plankton Diatom *Chaetoceras*, much resemble those of Chrysophyceae (Pascher, 1921, p. 244). In *Ophiocytium* (Fig. 2 *H*), *Tribonema*, and probably other members of Heterokontae the cell-walls consist of numerous thimble-like segments (Bohlin, 1897); the same structure is repeated in the envelopes of *Dinobryon*, *Hyalobryon*, and other Chrysophyceae (Pascher, 1921, p. 239) and is paralleled in those Diatoms in which intercalary bands in considerable numbers are intercalated between the valves and their connecting bands (cf. West and Fritsch, 1927, p. 339), a particularly striking example being furnished by *Rhabdonema*. Pascher (1914, p. 147), on the basis of these general resemblances, unites the three classes into a common phylum, Chrysophyta, which he regards as parallel with the Isokontae (his Chlorophyta); the Bacillariales are deemed to occupy a somewhat analogous position in the Chrysophyta to that of the Conjugatae among Chlorophyta. Such views are stimulating, but the classes of Pascher's Chrysophyta are still too imperfectly known to enable one to feel sure that the resemblances are of real significance. The Bacillariales offer a sharper contrast to the other Chrysophyta than do the Conjugatae to other Isokontae.

It will be familiar that the Diatoms naturally fall into two main series, Centricae and Pennatae, distinguished by their different symmetry, by the frequent motility of the individual in the latter series (a feature always associated with the presence of a raphe in the valve), and especially by their reproduction. For, whereas among Pennatae the auxospores are clearly originally the outcome of a conjugation-process (although now often produced apogamously, cf. Karsten, 1900), in the Centricae they are formed by rejuvenescence of the protoplasts and there is no indication that the process is sexual in any way. Moreover, in recent years evidence has been accumulating (cf. Pavillard, 1910, p. 542), for the view that the centric Diatoms possess a process of sexual reproduction, quite different from that of the Pennatae. Earlier records of the formation of special reproductive cells in marine Centricae are afforded by Murray (1896) and Gran (1902, pp. 23, 174), but general attention was first directed to these structures by Karsten (1904), who described in *Corethron* the production of numerous naked microspores which he suggested were gametes; other structures present were regarded as zygotes. Subsequently Bergon (1907), in *Biddulphia mobiliensis*, recorded similar microspores liberated as naked swimmers with two laterally attached cilia, whilst somewhat different swimmers were reported in a species of *Coscinodiscus* by Pavillard (1914). Gran (1904, p. 536) had previously recorded non-motile microspores of two sizes in *Chaetoceras decipiens* (cf. also Henckel, 1924). In *Melosira varians* Schmidt (1923) observed biciliate swimmers, four or eight of which were formed in the cells; other quadriciliate swimmers were regarded as zygotes, but no fusion was seen.

The data are not adequate for a clear oversight, but the sexuality of the microspores seems to be established (Oltmanns, 1922, p. 193; Karsten, 1924, p. 116). Karsten (1904, p. 553), in *Corethron*, produced some evidence for the occurrence of reduction during the germination of his putative "zygotes"; these gave rise to two individuals, each with two nuclei, one of which subsequently degenerated, a series of events strikingly like that seen in the germination of the zygospores of Desmidi. In Pennatae, however, there is a reduction in chromosome-number prior to sexual fusion, the ordinary pennate Diatom thus being diploid. Karsten's observations on *Corethron*, if true of centric Diatoms generally, would indicate that in them the individual is haploid. This contrast, added to the other points of difference between the two Diatom-series, has led to the view (Oltmanns, 1922, p. 194) that their resemblances may be the result of homoplasy and not imply any close relationship at all. Recently, however, Schmidt (1927) believes to have found in *Biddulphia sinensis* evidence of the diploid character of the individual and the haploid nature of the microspores. Neither Karsten's nor Schmidt's conclusions are, however, fully substantiated and, until this matter is cleared up, it is impossible to gauge the extent of the contrasts between Centricae and Pennatae. In relation to Pascher's hypothesis of a relationship between Bacillariales and Heterokontae, the cytology of those members of the latter group that are known to exhibit sexuality should be investigated.

The habit of Diatoms shows little diversity. The majority of the genera are unicellular, although many colonial forms are known, and it is of interest to note

that many of these are filamentous (*Melosira*, *Fragilaria*, etc.), whilst others repeat the dendroid type of colony found in other classes (*Gomphonema*, *Licmophora*).

#### 5. CRYPTOPHYCEAE.

The Cryptophyceae (Pascher, 1914, p. 158) include the flagellate Cryptomonadales and a small number of algal types. Unless, however, many of the latter are still to be discovered or have become extinct, it would appear that this class has passed but little beyond the confines of flagellate organisation. The typical motile unicell, as exemplified in the Cryptomonadaceae, exhibits a pronounced dorso-ventral construction with prominent flattening in the dorso-ventral plane, so that the cross-section is oval or elliptical (Fig. 4 A). When resting on one of its broader faces the individual exhibits a sloping truncate or emarginate front end, over which passes a more or less longitudinal furrow, running obliquely over the flanks to die out sooner or later without reaching the posterior end. The furrow appears as a slight notch at the front end. In most Cryptomonadaceae a tubular gullet extends from the anterior end of the furrow more or less deeply into the protoplast; this is readily seen in *Cryptomonas* (Fig. 4 A) and the colourless *Chilomonas*, and according to Zimmermann (1923, p. 285; 1924, p. 4; cf. also Karsten, 1898) is found also in *Rhodomonas* (Fig. 4 C) and *Chroomonas* (Zimmermann, 1924, p. 8), so that it would be absent only in *Cryptochrysis* (Fig. 4 G; Pascher, 1911, p. 190). The furrow or the gullet, as the case may be, is lined with small glistening bodies which appear to be of the nature of trichocysts (Fig. 4 C, t). Two<sup>1</sup> slightly unequal, somewhat band-shaped cilia arise either from the furrow or from the ventral edge of the gullet (cf. Fig. 4 G). According to Pascher (1911 a, p. 194) the majority of the Zooxanthellae belong to this type of Cryptomonadineae.

In a second family, the Nephroselmidaceae (Pascher, 1912 c), with *Protochrysis* (Fig. 4 B; Pascher, 1911, p. 191), *Nephroselmis*, and possibly *Sennia* (= *Nephroselmis* Senn, 1911, p. 643), the individuals are kidney- or bean-shaped, with the two cilia attached laterally, a little below the middle of the concave edge, the oblique furrow here running more or less transversely from the point of origin of the cilia (Fig. 4 B); in *Nephroselmis* there is a gullet. A prominent eye-spot (s, not so far recorded in Cryptomonadaceae) is situated just below the point of attachment of the cilia, one of which is directed forwards and the other backwards. There is an unmistakable resemblance between these Nephroselmidaceae and the typical swarmer (Fig. 4 F) of Phaeophyceae (Pascher, 1911 a, p. 198), but the resemblance appears to extend only to morphological features, and there is no clear evidence of any similarity in pigments or products of assimilation; nor is there in the Phaeophycean swarmer, as far as I am aware, anything to correspond to the furrow or in particular to the gullet of *Nephroselmis*. Pascher (1914, p. 153) is therefore probably right in his later view that these resemblances are of no phylogenetic significance, although the possibility of a distant relationship between the Nephroselmidaceae and the flagellate ancestors of Phaeophyceae (cf. below) may be kept in sight.

In both families there are usually two large parietal chromatophores (one in

<sup>1</sup> According to Pascher 1914, p. 150) some Cryptomonadales have only one cilium.



*Rhodomonas*, Fig. 4 C), although in a few cases they are numerous and discoid (*Cryptochrysis polychrysis* Pascher). The pigmentation is very varied (Pascher, 1911 a, p. 195), although olive-green and brown shades are the commonest. The deep blue-green colour of *Chroomonas* and the red of *Rhodomonas*<sup>1</sup> have in the past been taken as indicative of an affinity with the blue-green and red Algae respectively, but the forms in question are quite typical members of Cryptomonadaceae. Even though the red colour of *Rhodomonas* is due to phycoerythrin and is accompanied by a compound similar to Floridean starch, there is nothing otherwise to support an affinity with Rhodophyceae (Zimmermann, 1924, p. 9). For the rest, little is known as to the nature of the pigments in Cryptophyceae, although Pascher (1913 a, p. 96) suggests that they are similar to those of Peridinieae. Pyrenoids are usually present, but frequently lie apart from the chromatophores in the middle of the cell (Fig. 4 A, p). The products of assimilation collect as solid granules around the pyrenoids, as well as on the inner surfaces of the chromatophores; in some cases they consist of starch (species of *Cryptomonas*), whilst in others iodine produces a reddish or reddish-violet tint. Cysts with a cellulose membrane are known in certain species.

The Cryptomonadales appear as a very specialised group which, according to Pascher (1914, p. 150; 1913 a, p. 98), is more abundantly represented in the sea than in fresh water (cf. however Schiller, 1925 a, p. 194). Its origin is uncertain, although a resemblance to Ochromonadales has been pointed out (Pascher, 1911 a, p. 201) which may prove to have some significance.

Neither motile nor dendroid colonies are known in this class, but the palmelloid type (Phaeocapsales) is represented by *Phaeococcus* (Borzi, 1892, p. 463), *Phaeoplax* (Fig. 4 E; Pascher, 1911 a, p. 197; = *Phaeococcus marinus* Reinisch, 1911, p. 77)—the latter with swimmers (Fig. 4 D) resembling a *Cryptochrysis*—and perhaps *Naegeliella* (Correns, 1892) and *Phaeocystis* (Lagerheim, 1896). None of these, however, are properly known. Pascher (1914, p. 160) has also briefly described a chlorococcoid member of this class (*Tetragonidium*), the only genus of the Cryptococcales. This, which was found in moorland pools, has tetrahedral cells (12–18  $\mu$  in diameter) with cellulose walls, a brown lobed chromatophore with a pyrenoid, starch as the assimilatory product, a large excentric nucleus, and reproduction by *Cryptochrysis*-like zoospores. Filamentous forms are as yet unknown among Cryptophyceae. Sexual reproduction has been recorded only in *Phaeococcus*.

#### 6. DINOPHYCEAE (PERIDINIEAE).

This important class of marine and freshwater plankton organisms has, like the Chrysophyceae, been shown in recent years to possess a pronounced development in the direction of "algal" organisation. The motile Dinoflagellata show a construction which stamps them as relatively advanced forms (Fig. 4 L). The protoplast is provided with two furrows, the one transverse and the other longitudinal, and the same two furrows appear in the complex envelopes of the more specialised

<sup>1</sup> According to Zimmermann (1924, p. 5) the colour of *Rhodomonas* varies with the intensity of illumination.

types (*Peridinium*, *Ceratium*, etc.). At the point where the furrows meet there arise two cilia: one, taking the form of a narrow undulating band, occupies the transverse

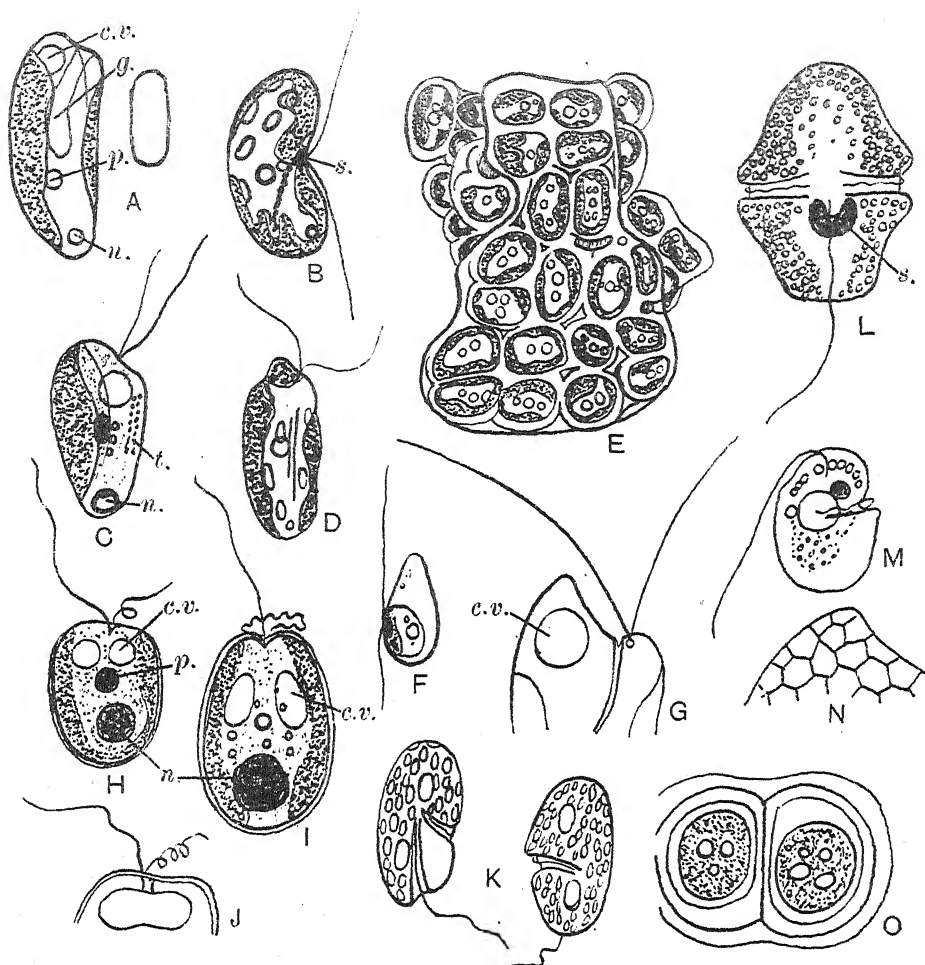


Fig. 4. A-E, and G, Types of Cryptophyceae. A, *Cryptomonas compressa* Pascher (after Pascher), without cilia; on the right the cell in section. B, *Protochrysis phaeophycearum* Pascher (after Pascher). C, *Rhodomonas lacustris* Pascher and Ruttner (after Ruttner). D, E, *Phaeoplax marinus* (Reinisch) Pascher (after Reinisch); D, swarmer; E, palmelloid colony. G, *Cryptochrysis commutata* Pascher, enlarged anterior end (after Pascher). F, *Ectocarpus*, zoospore (after Kuckuck, from Oltmanns). H-J, Types of Desmokytiaceae. H, *Haplodinium antjoliense* Klebs (after Klebs); J, front end of same enlarged (after Klebs). I, *Exuviaella marina* Cienk. (after Klebs). K-O, Types of Dinophyceae. K, *Hemidinium nasutum* Stein (after Schilling); on the left the front, on the right the back view. L, *Glenodinium cinctum* Ehrenb. (after Schilling). M, *Bernardinum bernardinense* Chod. (after Chodat). N, *Gymnodinium hiemale*, small part of membrane (after Woloszynska). O, *Gloeodinium montanum* Klebs, palmelloid form (after Klebs). c.v. contractile vacuoles; g. gullet; n. nucleus; p. pyrenoid; s. stigma; t. trichocysts.

furrow which is usually well marked and, except in *Hemidinium* (Fig. 4 K) and the colourless *Bernardinum* (Fig. 4 M; Chodat, 1923, p. 40), completely encircles the

cell; the second, more thread-like, cilium is directed backwards during movement, its proximal portion occupying the longitudinal furrow, which is not always clearly defined, whilst the longer distal portion projects into the surrounding water (Fig. 4 *L*). In the protoplast one can generally distinguish an outer denser and somewhat granular region harbouring the usually numerous and more or less discoid chromatophores, whilst the nucleus occupies the inner more vacuolate portion. The pigmentation of the chromatophores is diverse, yellow, brown, and reddish colours being the predominating ones<sup>1</sup>; various pigments have been extracted from them (Schütt, 1890; Molisch, 1923, p. 259). Reserve-food is stored as starch and as oil, which is often yellowish or reddish. Pyrenoids have been recorded in a few forms (Geitler, 1926). The nucleus is usually large and conspicuous, being either finely granular or containing numerous fine chromatin-threads (Klebs, 1912, p. 416).

While the majority of the Dinoflagellata possess a complex membrane consisting in the main of cellulose (Mangin, 1911) and composed of a definite number of regularly arranged and often elaborately sculptured plates (*Peridinium*, *Ceratium*, etc.), there are a certain number in which there is only a delicate membrane (*Hemidinium*, *Glenodinium*, Fig. 4 *L*) or in which the individuals appear to be naked (*Gymnodinium*, cf. especially Kofoid and Swezy, 1921). Various observations seem to show, however, that, even in these cases, a membrane is (always?) present and that it is composed of a number of equal polygonal plates (Fig. 4 *N*; West, 1916, p. 61; Woloszynska, 1917, p. 114; Lindemann, 1926).

Although temporary colonies are formed in some Dinoflagellata through failure of the dividing individuals to separate, *Polykrikos* is the only motile colonial type so far recorded. Only one palmelloid form (Dinocapsales) is known, viz. *Gloeodinium* (Fig. 4 *O*; Klebs, 1912, p. 411; Killian, 1924; Reichardt, 1927; Pascher, 1927 *a*, p. 45), a moorland Alga with packets of rather large cells embedded in stratified mucilage and showing the typical structure of the Peridinian cell, except for the absence of furrows. Killian records reproduction by swarmers resembling a *Hemidinium*, although his figures are not very convincing. Pascher (1927 *a*, p. 46) speaks of other freshwater and marine Dinocapsales, without giving details.

Since Klebs' (1912) important memoir on the alga-like Peridiniae, chlorococcoid organisation has been a familiar feature of the class<sup>2</sup> and our knowledge of these forms (Dinococcales) has been appreciably extended by Pascher (1927 *a*). As in the Chlorococcales, some Dinococcales propagate by zoospores, whilst others exhibit no motile reproductive cells, but the different habit appears here to be in general only a specific feature. Several species of Klebs' *Cystodinium* (Fig. 5 *F*), with more or less lunate cells whose tips are often markedly produced, reproduce by *Gymnodinium*-like zoospores (Fig. 5 *I*), formed to the number of two (Fig. 5 *H*) or four by division of the contracted protoplast of the cell, which at this stage and

<sup>1</sup> Reddish tints are more frequent in marine Peridiniae. A few blue-green forms have been recorded (cf. Geitler, 1924 *a*, p. 359).

<sup>2</sup> The writer is unable to agree with Kofoid and Swezy (1921, p. 109) that Klebs' forms "present none of the dinoflagellate characteristics."

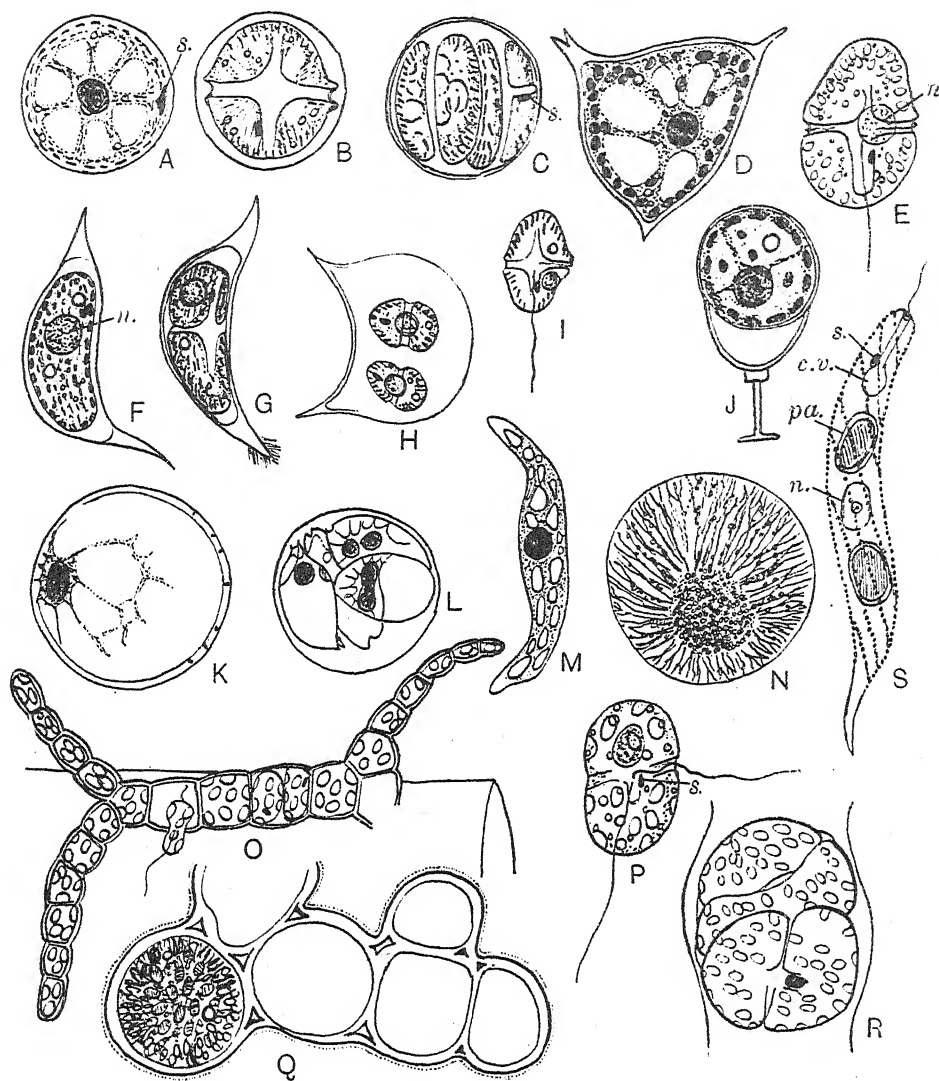


Fig. 5. A-R, Types of algal Dinophyceae. A-C, *Hypnodinium sphaericum* Klebs (after Klebs); B, incipient division; C, division into two daughter-cells. D, E, *Tetradinium minus* Pascher (after Pascher); E, swarmer. F-I, *Cystodinium steinii* Klebs (after Klebs); G, incipient division; H, late stage; I, swarmer. J, *Stylodinium globosum* Klebs (after Klebs). K-M, *Dissodinium lunula* (Schütt) Klebs; K, chlorococcoid stage; L, formation of horned cysts; M, mature cyst (all after Dogiel). N, *Pyrocystis noctiluca* Murray (after Murray). O, P, *Dinoclonium conradi* Pascher, heterotrichous filament (after Conrad, from Pascher); P, swarmer. Q, R, *Dinothrix paradoxa* Pascher (after Pascher); R, cell with two swarmers. S, *Euglena spirogyra* Ehrenb. (after Lemmermann). c.v. contractile vacuole; n. nucleus; pa. paramylon-grain; s. stigma.



prior to division develops the characteristic furrows (Fig. 5 G), although these are not discernible in the uncontracted protoplast. Pascher (1927 a, p. 37) has, however, described other species of the genus (e.g. *C. lunare*) in which zoospores are suppressed and furrows never appear at any time in the protoplast.

Those forms of *Cystodinium* that reproduce by swimmers are manifestly not very far removed from species of the genus *Gymnodinium*, where likewise resting-stages are occasionally produced, except that the relative importance of the motile and sedentary phases in the life-cycle is reversed. In *Tetradinium* (Fig. 5 D), a parallel to *Tetraëdron* among the Isokontae, reproduction by zoospores (Fig. 5 E) appears to be usual, but the protoplast here develops no furrows prior to division to form the swimmers. A further step in the elimination of the motile phase is seen in *Hypnodinium* (Fig. 5 A-C), whose large spherical cells exhibit a contraction of the protoplast accompanied by furrow-formation (Fig. 5 B), after which division into two takes place (Fig. 5 C) without a motile phase; in this form a prominent eye-spot (s) is generally distinguishable in all stages. In *Phytodinium*, lastly, neither swimmers nor any indication of furrow-formation are found and the last traces of the motile ancestry have vanished. The Dinococcales also include the epiphytic *Stylodinium* (Fig. 5 J), a parallel to *Characium* among Chlorococcales, which probably reproduces by swimmers. A few other genera are known (cf. Pascher, 1927 a), and it seems probable that many more will come to light.

Klebs and Pascher both include among the Dinococcales the genera *Pyrocystis* (*P. noctiluca* Murray) and *Dissodinium*<sup>1</sup>. In the latter (cf. Dogiel, 1906, p. 4), a marine plankton organism, the normal vegetative condition is represented by a large vesicular cell within whose thin membrane is a huge vacuole traversed by delicate protoplasmic strands (Fig. 5 K). Within these cells there are formed sooner or later by progressive division (Fig. 5 L) as many as sixteen lunate cells (Fig. 5 M) resembling the sedentary stage of *Cystodinium*, and these develop further, as in that genus, with the liberation of a number of *Gymnodinium*-like swimmers from which the spherical cells presumably again arise. In *Pyrocystis* (Fig. 5 N), an abundant form in all tropical seas, this is the condition of the organism throughout life, neither cysts nor swimmers being formed and multiplication occurring merely by division of the contracted protoplast (cf. *Phytodinium*).

In 1914 Pascher briefly described a filamentous marine member of this class under the name of *Dinothrix*, of which he has recently given a full account (1927 a). In the same paper (p. 15) a short description and figure of a second genus of Dinotrichales, *Dinoclonium*, discovered by Conrad, is given. Both reproduce by zoospores resembling a *Gymnodinium* (Fig. 5 P). *Dinothrix* has little-branched threads composed of few barrel-shaped cells with stratified membranes (Fig. 5 G) and evidently represents a very lowly type of filament, reproducing abundantly by fragmentation. *Dinoclonium* (Fig. 5 O) is an epiphyte on other Algae and belongs to the heterotrichous filamentous type with prostrate and projecting threads, the

<sup>1</sup> This is the *Gymnodinium lunula* (later *Pyrocystis lunula*) of Schütt (1896, p. 3) which was made the type of the genus *Diplodinium* by Klebs (1912, p. 390), the name being later changed to *Dissodinium*.



latter unbranched and running out to a point. The occurrence of this type of body in this class is of great interest and suggests that we are as yet only at the commencement of our knowledge of the filamentous Dinophyceae. Possibly some of the little-known filamentous Phaeophyceae may prove to belong here.

Many authorities (Jollos, 1910, p. 203; Pascher, 1914, etc.; Kofoed, 1920) now regard the Cystoflagellata, and particularly *Noctiluca*, as a special offshoot of the Dinoflagellata. Many of the latter exhibit occasional holozoic nutrition, and *Noctiluca* would represent a type in which great specialisation in this direction has taken place. In some of its stages it resembles the holophytic *Pyrocystis*, and there has in the past been some confusion between the two organisms.

The preceding considerations will have shown that a considerable number of sedentary algal members of Dinophyceae are known; nevertheless, as in Chrysophyceae, the motile unicell seems to be the most widely represented type. The question as to the nature of sexual reproduction in this class is still unsettled, but it seems to be of rare occurrence and isogamous. Zederbauer (1904) and Entz (1909) record a copulation between the protoplasts of two individuals in *Ceratium*, observations that have been questioned by various subsequent writers. Hall (1925) again describes binucleate cysts in *C. hirundinella* and supposes that they have resulted from sexual fusion. Pascher (1914, p. 152; 1927 a, p. 34) refers to the formation of small isogamous swimmers, having the structure of a *Gymnodinium*, in *Hypnodinium* (which in its asexual propagation shows no motile stages, cf. above); the resulting thin-walled zygote is stated to grow slowly until it reaches the size of the ordinary vegetative cell, whose contents subsequently divide to form four swimmers. These observations scarcely fall into line with what is known of the ordinary reproduction of this genus. It should be added that Pascher (1914, p. 152) mentions the occurrence of similar small swimmers in other Peridinieae.

*Desmokontae*. Pascher (1914; 1927 a) has grouped under this name a number of forms with yellowish or brownish chromatophores that are usually brought into more or less close relation with the Peridinieae, and it will be well to consider them first before discussing the affinities of Dinophyceae. The simplest member of these Desmokontae is *Desmomastix* (Pascher, 1914, pp. 148, 160), a freshwater form of which at present only a brief description is available. Its naked ellipsoidal cells (with a circular cross-section) possess a large chromatophore with an excentric pyrenoid; the assimilatory product is starch. There are two apical, band-shaped cilia, one of which exhibits undulatory movements, resembling those of the transverse cilium of Peridinieae. Spherical cysts with a cellulose membrane are recorded. In the marine *Pleromonas* (Pascher, 1914, pp. 148, 159) there is a faint incision at the front end, and it is from this that the two cilia arise; these resemble those of *Desmomastix*, but the undulatory cilium is directed more or less horizontally. The cells of *Pleromonas* are enveloped in a delicate cellulose membrane which under certain treatment tends to break into two irregular valves.

A rather better known form is *Haplodinium* (Fig. 4 H; Klebs, 1912, pp. 370, 438) which is at present recorded only from brackish tropical waters. Its broadly ovate, somewhat flattened cells, provided with a firm cellulose membrane, possess a

slightly oblique truncated front end with a faint incision from which the two unequal cilia arise (Fig. 4 J); one of these is stretched out anteriorly, while the other is coiled in cork-screw fashion and directed more horizontally. There are two plate-like yellowish chromatophores, each with a projecting pyrenoid, whilst a large nucleus (Fig. 4 H, n) lies at the back and two large (contractile?) vacuoles at the front end. The three forms just considered are regarded as closely allied (cf. also Oltmanns, 1922, p. 53), but *Desmomastix* and *Pleromonas* are so imperfectly known that, especially as regards the former, most will feel inclined to await further investigations. To the same series belongs *Entomosigma* (Schiller, 1925 a, p. 195).

Oltmanns includes these genera in Prorocentraceae, with which *Haplodinium* certainly appears closely related (cf. also Klebs, 1912, p. 372). In *Exuviaella* (Fig. 4 I; Klebs, 1884, p. 743) and the strongly flattened *Prorocentrum*, both of which are marine, we have forms with the same general construction as *Haplodinium*, except that, as in *Pleromonas*, the cell-membrane is composed of two halves, here provided with numerous fine pores. Pascher and Oltmanns agree in regarding the Dinophysidaceae, which are mainly found in tropical seas, as derived from forms similar to the Prorocentraceae. They likewise possess a membrane composed of two practically equal valves, but the cells are provided towards the anterior end with a pronounced transverse furrow harbouring the horizontal cilium.

Pascher (1914, p. 156) groups all these diverse motile unicellular forms as Desmomonadales, and in the same place (pp. 149, 160) briefly describes a marine palmelloid member of this series (*Desmocapsa*) which is stated to reproduce by swarmers resembling *Desmomastix*.

Pascher (cf. also 1927 a, p. 50) holds that Desmokontae, Cryptophyceae, and Dinophyceae represent three divergent lines from a common stock and groups them in the phylum Pyrrophyta, equal in rank with the Chlorophyta and Chrysophyta (cf. p. 121). In his words (1914, p. 148): "It appears that side by side with the closely related flagellate series Cryptomonadales and Dinoflagellata there exists a third group of Flagellates, which however by contrast to the two former, which are certainly derived forms, gives in its simplest members a much more primitive impression," and again (p. 149): "The Cryptomonadales are probably derived from the Desmomonadales with special development of the dorsiventrality and the furrow-structure" which latter is regarded as being foreshadowed by the apical incision of *Pleromonas* and *Haplodinium*. With this suggestion of affinities one is quite prepared to agree, but it should be noted that we know nothing as to the meaning of the furrow-formation; it may not be fundamental, and maybe stress is being placed on a character of no great importance. Even without this, however, there is sufficient resemblance between *Haplodinium* and the Cryptomonads to warrant the assumption of a considerable degree of relationship.

There is not so much to be said for the direct affinity of the Desmomonadales and Dinophyceae, but an agreement between the latter and Cryptomonadales is clear and has been pointed out by several authorities. The simpler Dinoflagellata are profitably compared with the Nephroselmidae (p. 123); in particular such a form as *Hemidinium* (Fig. 4 K) shows considerable resemblances to *Protochrysis*

(Fig. 4 B). We may well be dealing with a large plexus of brown, more or less closely related types, but too many of the significant genera are imperfectly known to be able to decipher the affinities clearly. Few would now wish to support the view of a relation between Dinophyceae and Bacillariales.

#### 7. CHLOROMONADALES.

This small group, comprising the genera *Vacuolaria*, *Trentonia*, *Gonyostomum*, and a colourless form, *Thaumatomastix*, all with obscure affinities, is of no special interest from the present point of view (cf. Pascher, 1913 a, p. 175).

#### 8. EUGLENINEAE.

This class includes only flagellate types, no algal forms being at present known, and the members show a more definite trend in the direction of animal organisation than is found in most of the classes of pigmented Protophyta. In fact, in many members of the colourless Peranemaceae holozoic nutrition is the rule and the organisms are specially equipped for the purpose. It is possible that the future may show the existence of "algal" derivatives, but all the known Euglenineae (*Euglena*, *Phacus*, *Lepocinclis*, etc.) are so specialised that this appears little likely. The chromatophores are usually several or numerous and in colour do not differ from those of the ordinary green plant, although as far as the writer is aware nothing is known as to the detailed nature of the pigments. The characteristic assimilatory product is the polysaccharide paramylon (Bütschli, 1906); it appears in the shape of rods, discs, or rings (Fig. 5 S, pa) which in many cases possess a great degree of persistence and constancy of type, so as to be of value for specific diagnosis. Bodies of the nature of pyrenoids occur in the chloroplasts in some cases. The periplast is often soft admitting of considerable change of shape, although many Euglenineae have firm periplasts and a practically constant outline. The periplast is invaginated at the anterior end (Fig. 5 S) to form a short narrow canal which leads down to a prominent contractile vacuole into which a system of accessory vacuoles discharges. Most Euglenineae possess a single coarse flagellum arising from the canal (two in *Eutreptia*).

The usual method of reproduction is by longitudinal division. There are but few records of a sexual process. A fusion of amoeboid gametes has been described by Haase (1910, p. 52) in *Euglena sanguinea*, whilst Dobell (1908, p. 75) records a copulation of ordinary individuals in *Scytomonas pusilla* Stein (*Copromonas subtilis* Dobell).

The affinities of this specialised class are altogether obscure, but in spite of its limited development it is of interest to notice that parallelism with other classes of Protophyta is in so far displayed as, apart from the motile unicell, we find among Euglenineae the encapsuled type (*Trachelomonas*) and the dendroid colony (*Colacium*).

#### 9. PHAEOPHYCEAE.

The brown Algae are sharply characterised by a whole series of distinctive features, among which the type of swarmer stands prominent. The two laterally

placed cilia, attached rather below the middle of the body, one directed forwards and the other backwards, the large conspicuous eye-spot at the point of insertion of these cilia, and the not uncommon presence of a single chromatophore located at the same point (cf. Fig. 4 *F*), are features that are quite unlike those of the ordinary motile type in other classes of Protophyta and indicate an origin for Phaeophyceae from a flagellate ancestry possessing these particular characteristics. True, aberrant types of swimmers are found in a few cases (Dictyotales), but these exceptions are few and on the whole the swimmer in Phaeophyceae is more constant than in many other classes of Protophyta. The brown colour is due to fucoxanthin, a carotinoid pigment, which accompanies the usual chloroplast-pigments (Willstätter and Page, 1914<sup>1</sup>); bodies which are possibly of the nature of pyrenoids are found in some of the less specialised forms. The products of assimilation are stored, according to Kylin (1918, p. 16), in the form of polysaccharides (laminarin), mannite, and fat. The cell-contents commonly include so-called fucosanes, about the nature of which there have been many differences of opinion (cf. Kylin, 1918; Molisch, 1923, p. 391), but which appear in the main to contain tannins. Little is known as to the composition of the cell-membranes in this class, although usually mucilaginous and containing various pectic substances. There can be no doubt, however, that the brown Algae possess a type of metabolism distinct from that of other Protophyta.

In view of the widespread occurrence of zoospores and motile gametes, it cannot be doubted that the Phaeophyceae originated from motile Flagellata with the characters above indicated, but as far as present knowledge goes these are altogether extinct. As explained on p. 123, there are certain resemblances between the Phaeophycean swimmer and the Nephroselmidae among Cryptomonadales which may indicate a remote affinity, but not a direct relationship. The antiquated view of an affinity between Chrysomonadales and Phaeophyceae, which still exists in certain quarters, altogether overlooks the pronounced differences in nearly every respect between the motile cells in the two classes.

The simplest known members of the Phaeophyceae, represented by the Ectocarpaceae of the group Ectocarpales, are fairly advanced filamentous forms, parallel to the Chaetophorales among Isokontae. *Ectocarpus* and others exhibit the heterotrichous filamentous type and, here as there, we have a reduction-series culminating in discoid forms (*Ascocyclus*, cf. especially Oltmanns, 1922 *a*, p. 6 *et seq.*). But the vast majority of the Ectocarpales and the other groups of brown Algae exhibit a much more elaborate construction and attain to marked external and internal differentiation. These are not to be classed as Protophyta, if indeed even an *Ectocarpus* can justifiably be so called, and are thus beyond the scope of this article. Attention may be drawn to the fact that sexuality is clearly established for most of the simple Ectocarpaceae and, although in some cases isogamous, betrays a very marked tendency towards anisogamy or oogamy.

<sup>1</sup> Molisch (1923, p. 256) holds a somewhat different view.

## 10. RHODOPHYCEAE.

The red Algae, apart from their numerous other peculiarities, are essentially characterised by the lack of ciliated reproductive cells<sup>1</sup>. All the latter (monospores, tetraspores, carpospores, spermatia) are motionless, although slight amoeboid movements have been recorded in a few cases and Rosenvinge (1927) has recently given an account of slow gliding movements whose mechanism is unknown and which may possibly be of widespread occurrence. The question at once arises whether the ancestry of the class is to be sought among motile Flagellata at all, but to this question there is no answer that can be based on any definite facts. There is, however, no valid reason why we should suppose that the ancestors must of necessity have been motile.

Physiologically the class is distinguished by the presence in the chromatophores of phycoerythrin and phycocyanin in varying proportions, side by side with chlorophyll, etc. (Kyllin, 1912), by the formation of solid storage-products in the form of so-called Floridean starch (Molisch, 1923, p. 388), and by the possession of relatively thick mucilaginous membranes commonly provided with large pits occupied by conspicuous protoplasmic strands. Pyrenoid-like bodies, while of frequent occurrence in the Nemalionales, are very rare in the higher Florideae.

There is no evidence of affinity with other classes of Protophyta. *Rhodomonas*, although possessing similar pigments and assimilatory products, exhibits no other points of agreement, and it is impossible to believe in a relationship until other points of contact have been found. The separate origin of the class is almost beyond question.

The two series of red Algae, Bangiales and Florideae, are possibly but little related to one another. The former, characterised by their axile, more or less stellate chromatophores with prominent pyrenoids and the absence of pits in the cell-walls, show very simple types of bodies with practically no differentiation (*Bangia*, *Porphyra*); they also include a few reduced types (*Porphyridium*, cf. Geitler, 1924 a, p. 362). The resemblances in methods of reproduction with the lower Florideae (Nemalionales) are not very profound and possibly due to convergent development. The lowest members of the Florideae again include types with heterotrichous filaments, but the vast majority exhibit a higher differentiation, rivalling, but not quite equalling, that of the brown Algae. In the complexities of their life-cycle they are more specialised than any other class of Algae.

## 11. MYXOPHYCEAE (CYANOPHYCEAE).

Like the Rhodophyceae, the blue-green Algae are not at present known to possess any swimmers. *Chroomonas* which, owing to its blue-green colour, was at one time regarded as a motile unicell belonging to this class, has far too elaborate a cell-structure to come into consideration. An old record of zoospores in *Merismopedia* has never been confirmed. We are of course confronted with the same alternatives as in the red Algae, but in the Myxophyceae a somewhat better case can

<sup>1</sup> Yendo (1919) recorded motile gametes in the Bangiales, but this has been discredited by subsequent workers.



be made out for the view that they have never possessed motile stages. The prevalent terrestrial tendency of the class is significant, also the absence of all sexual reproduction; except in the Chamaesiphonales, there are no sporangial structures. As in Diatoms and Conjugatae, too, other methods of movement (hormogonia) have been acquired. All the members of the class are algal.

An affinity with other series of pigmented Protophyta appears out of the question, despite the occurrence in Myxophyceae of phycocyanin and phycoerythrin, the typical accessory pigments of Rhodophyceae (Boresch, 1921; Wille, 1922). These are, however, apparently accompanied solely by chlorophyll, and the yellow carotin and all four substances are diffused through the peripheral cytoplasm without differentiation of a definite chromatophore. The products of photosynthesis are glycogen (or a glycoprotein, cf. Baumgaertel, 1920, p. 98), while as a general rule actively assimilating cells contain many small, bright, highly refractive granules which appear to be protein in nature (cyanophycin granules). No exact parallel for either substance seems to be found in other classes of pigmented Protophyta. A further peculiarity of the Myxophyceae lies in the central body, but this is not the place to enter into a discussion of the many different views that have been held as to its nature and structure (cf. West and Fritsch, 1927, p. 437). Whatever the opinion as to details may be, all will agree that the cell-structure is very simple, far simpler than that of any other class of Protophyta here considered. The cell-membrane, about the nature of which there exists much uncertainty, seems to be very different from that of other Algae, alone in its intimate connection with the protoplast, while the absence of any evident vacuoles in the latter except in moribund cells is a further distinctive feature (cf. Brand, 1903).

In striking contrast to the simple cell-structure and simple morphological construction of the class, is its exceedingly wide distribution and the adaptation of its members to a great variety of situations (West and Fritsch, 1927, p. 434). The only indication of specialisation lies in the heterocysts found in most of the filamentous groups and probably representing some reproductive structure that has now become largely functionless (Geitler, 1921). It is probable that the class is of great antiquity (cf. Pia, 1924, p. 175) and has retained many primitive features.

The Myxophyceae fall naturally into three groups. The Chroococcales include unicellular and colonial forms, parallel with the Chlorococcales, etc., and in part developing very similar forms of colonies. The filamentous type (Hormogoneales) exhibits very varied differentiation, with simple and branched threads, although true branching is found only in a small number of forms. The Chamaesiphonales are a specialised group whose relation to Chroococcales is not clear. They include unicellular and filamentous forms, the latter (Pleurocapsaceae) showing heterotrichous filaments in which the basal system is often parenchymatous, whilst the upright system frequently consists of densely aggregated threads, so that the filamentous habit is obscured and a colonial one suggested (Geitler, 1925, p. 238).

## 12. COLOURLESS PROTOPHYTA.

Mention has been made in the preceding matter of the existence in many classes of colourless genera that are quite plainly related to the pigmented types. Among Isokontae such colourless derivatives can be recognised in every subdivision of Volvocales and Chlorococcales (cf. Fig. 6 A-C and Wille, 1909; Printz, 1927). *Chilomonas* is a colourless member of Cryptomonadaceae, *Oxyrrhis* (Fig. 6 I; Senn, 1911, p. 606) a colourless Dinoflagellate<sup>1</sup>, etc. In many series (Chrysophyceae,

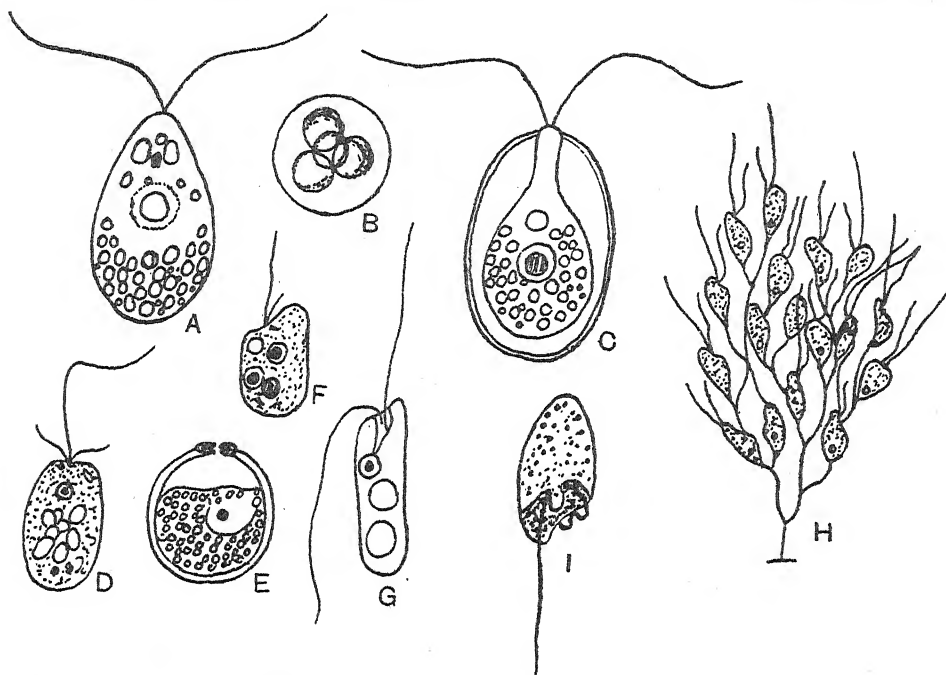


Fig. 6. Types of colourless Protophyta. A, *Polytoma ocellatum* Francé (Isokontae, after Francé). B, *Mycacanthococcus cellularis* Hansg. (Isokontae, Chlorococcales, after Hansgirg). C, *Chlamydo-blepharis brunnea* Francé (Isokontae, after Francé). D, *Monas vivipara* Ehrenb. (after Lemmermann) (Protomastiginae, Chrysophyceae?); E, cyst of same (after Prowazek). F, *Oicomonas ocellata* Scherffel (after Scherffel, Protomastiginae, Chrysophyceae?). G, *Phyllomitus amylophagus* Klebs (after Lemmermann) (Protomastiginae, Cryptophyceae?). H, *Dendromonas laxa* (Kent) Blochm. (after Kent) (Protomastiginae, Chrysophyceae?). I, *Oxyrrhis marina* Duj. (after Senn) (Dinophyceae).

Bacillariales, Dinophyceae, Euglenineae, etc.) occasional colourless species of pigmented genera are known. Pascher (1917 a, p. 48) puts together a number of data indicating a gradual reduction in the size of the chromatophores in certain of these classes.

In addition a large number of separate families of colourless Flagellata are recognised, usually grouped (Senn, 1900) in the three series Pantostomatinae, Protomastiginae, and Distomatinae, of which the second is much the largest. While the Distomatinae include few and specialised forms, the other two series contain

<sup>1</sup> Cf. also the numerous parasitic forms described by Chatton (1920).

many relatively simple types. It has been shown by Scherffel (1911, p. 327) and Pascher (1912 a, p. 189) that various Protomastiginae are colourless Chrysomonadales; this is true of species of *Monas* (Fig. 6 D) and *Oicomonas* (Fig. 6 F), and is also stated to apply to the colonial *Anthophysa*, *Cephalothamnion*, *Stylobryon* (Pascher, 1917 a, p. 39), and *Dendromonas* (Fig. 6 H). The reference of these colourless Flagellates to Chrysomonadales is based mainly on the possession by them of the characteristic cysts (cf. Fig. 6 E), whilst in several cases leucosin is present as well. Various workers (cf. Pascher, 1911 b, p. 121) have pointed out the resemblance between Cyrtophoraceae (Chromulinales, cf. Fig. 7 L) and certain Pantostomatinae (*Actinomonas*, *Pteridomonas*, etc.). Pascher (1917 a, p. 39), without giving details, refers *Phyllomitus* (Fig. 6 G; Protomastiginae) to the Cryptomonadales, which appears very plausible, while *Furcilla* (Fig. 1 K), as already mentioned (p. 106), is now regarded as a colourless member of Chlamydomonadaceae. The Protomastiginae are thus beginning to crumble, and subsequent investigations will in all probability still further reduce their numbers.

In view of this very obvious tendency, especially in some series of pigmented Protophyta, to adopt a completely colourless habit with loss of plastids, one is tempted to suspect here an origin for such extensive groups of colourless plants as are constituted by the Phycomycetes, and especially perhaps to seek for analogies among the unicellular Chytridiales which habitually reproduce by swarmers. It must be realised, however, that in most cases the colourless Isokontae, etc., above mentioned, are very closely related to the respective pigmented types, and neither in their swarmers nor in other respects do they show any clear indications of affinity with Chytridiales. Alone, among the Chlorococcales, the series of space-parasites including *Chlorochytrium* and *Phyllobium* may by way of the colourless *Rhodochytrium* (Lagerheim, 1893) afford some basis for assuming such an affinity; there are some possible resemblances to forms like *Synchytrium* here (cf. however, Chodat, 1913, p. 250; Griggs, 1912, p. 161). Otherwise, however, there is no evidence, as far as the writer is aware, that any of the lower Fungi are connected with the many colourless derivatives of the simpler Protophyta. Many of the latter thrive where some organic nutriment is available, and this has no doubt led to the frequent adoption of a saprophytic habit (cf. also *Chloramoeba*, p. 113; *Euglena*, Zumstein, 1900; Ternetz, 1912; Wenrich, 1924).

It might be conjectured that the Fungi are derived from pigmented groups now extinct by loss of plastids and adoption of a saprophytic or parasitic habit, but such speculative hypotheses are quite futile. There is no *prima facie* reason at all why the Fungi should not have been colourless from the first, but a careful enquiry along these lines has not yet been undertaken (cf. however Scherffel, 1925). It is unlikely that all the Protomastiginae will prove to be colourless members of the pigmented classes, and a comparative study of the swarmers of Chytridiales and Phycomycetes may betray a direct relation between them and some of the large residue of colourless Flagellata. There are probably quite a number of different types hidden among the latter, and some of them may be the motile unicells of classes in which perhaps certain Chytridiales represent the chlorococcoid types and

certain Phycomycetes the filamentous members. This is a pure hypothesis, but it may be worthy of investigation by those better acquainted with the lower Fungi than I am. There may be no basis for it, but seeing that recent research has shown that there is a strong filamentous tendency in many classes of pigmented Protophyta, it is at least possible that the same holds for some of the colourless series.

Many authorities have believed that the filamentous Fungi are more directly related to the filamentous Algae. The Monoblepharidaceae are now regarded by many as colourless members of Siphonales (Printz, 1927, p. 252), although previously included among Phycomycetes. The strangely isolated Vaucheriaceae show points of contact with certain groups of the latter (West and Fritsch, 1927, p. 293) which may, however, be due purely to convergent development. These very scanty data afford no good foundation for a direct relation between filamentous Isokontae and Phycomycetes, and the "evidence" for the relation of higher Fungi to Florideae appears to have even less basis.

### 13. AMOEBOID AND RHIZOPODIAL TENDENCIES AMONG PROTOPHYTA.

Largely as a result of Pascher's researches, it has become apparent during recent years that, especially in certain classes of Protophyta, there is a considerable tendency towards loss of cilia and adoption of an amoeboid or rhizopodial habit in conjunction with holozoic nutrition. Most of the data are summarised in Pascher's memoir *Flagellaten und Rhizopoden in ihren gegenseitigen Beziehungen*, published in 1917, of which only a brief résumé can be given. Pascher (1917 a, p. 10 *et seq.*) cites many instances of the assumption of an amoeboid habit with protrusion of relatively blunt pseudopodia aiding in holozoic nutrition. Temporary amoeboid stages have been observed in the swimmers of various Isokontae (Fig. 7 B; Pascher, 1909, 1915 b, 1918; Puymaly, 1922), in many Chrysophyceae, Dinophyceae, etc. Even in so highly organised a form as *Mallomonas* such stages occur (Conrad, 1914). *Cryptochrysis amoeboides* (Pascher, 1917 a, pp. 18, 20) is a temporarily amoeboid Cryptomonad, and *Rhizochloris* (Fig. 7 A; *ibid.* p. 36) a similar member of Heterokontae.

Other such forms are colourless, their reference to the respective classes depending on the retention of certain characteristic features of the latter. Pascher (1917 a, p. 51) briefly refers to an amoeboid member of Chlamydomonadales, *Gametamoeba*, which produces biciliate gametes. *Leukochrysis* (Fig. 7 E-G) is a colourless amoeba that betrays its Chrysophycean affinities by the occasional production of the characteristic endogenous cysts (Fig. 7 F), while *Dinamoebidium* (Fig. 7 H; first described as *Dinamoeba*, Pascher, 1915 a, p. 118) is an amoeboid Dinophycean within whose cysts (Fig. 7 I) colourless *Gymnodinium*-like swimmers (Fig. 7 J) are produced. These various colourless types would all be regarded as species of *Amoeba*, in the absence of knowledge of their reproductive stages.

In other cases the protoplast is protruded into the long delicate and often branched processes, known as rhizopodia, and an exceptionally marked development of such forms is found among Chrysophyceae. Klebs (1893, p. 406) long ago described a Chrysomonad, *Chrysamoeba* (Fig. 7 C), which in certain stages has all

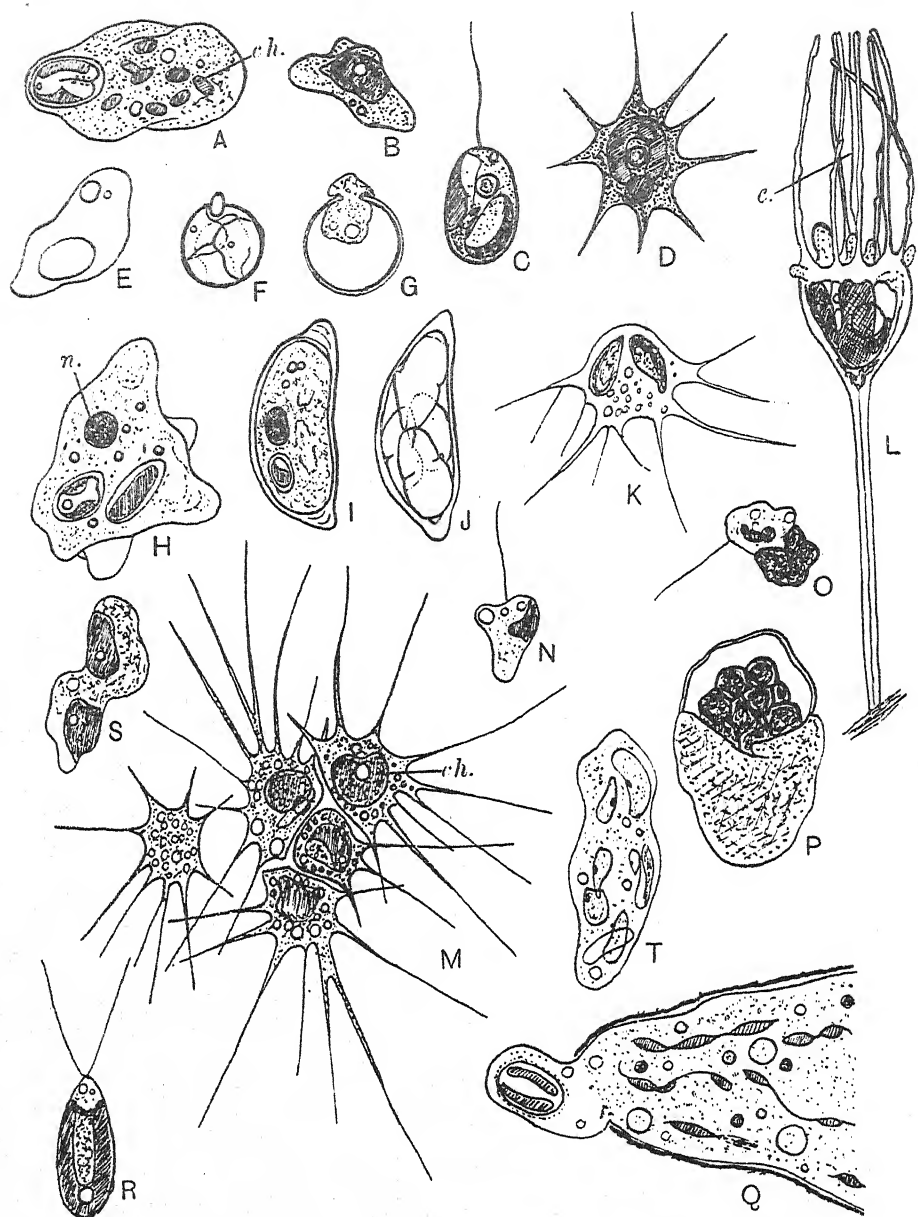


Fig. 7. Amoeboid and rhizopodial forms among Protozoa. A, *Rhizochloris mirabilis* Pascher (Heterokontae, after Pascher). B, *Aphanochaete pascheri* Heering, amoeboid zoospore (after Pascher). C, D, *Chrysamoeba radians* Klebs (after Klebs); D, in the rhizopodial stage. E-G, *Leukochrysis* (Chrysophyceae, after Pascher); E, amoeba; F, cyst; G, amoeba escaping from same. H-J, *Dinamoebidium varians* Pascher (Dinophyceae, after Pascher); H, amoeba; I, cyst; J, formation of swarmers. K, *Synura uvella* Ehrenb., rhizopodial stage (after Pascher). L, *Cyrtophora pedicellata* Pascher (after Pascher, Chrysophyceae). M, *Rhizochrysis scherffellii* Pascher (after Scherffel, Chrysophyceae), permanently rhizopodial. N-Q, *Myxochrysis paradoxa* Pascher (Chrysophyceae, after Pascher), plasmoidal form; N, swarmer; O, the same escaping from a cyst; P, formation of cysts; Q, plasmodium. R-T, *Chlamydomonas* sp.; R, ordinary individual; S, fusion of amoeboid gametes; T, plasmodium formed by fusion of the amoeboid zygotes (all after Pascher). c, cilium (in fig. L); ch, chromatophore; n, nucleus.



the characters of a *Chromulina*, but at other times loses its cilium and develops long unbranched rhizopodia (Fig. 7 D). Pascher (1917 a, p. 18) describes similar stages in a species of *Ochromonas*, whilst remarkable rhizopodial forms are occasionally assumed in *Synura* (Fig. 7 K; Pascher, 1912 a, p. 161; 1917 a, p. 21). *Heterochloris* (Fig. 2 B, C) furnishes an analogous example among Heterokontae. The occurrence of rhizopodia in some of the epiphytic Chrysophyceae was mentioned on p. 117. Rhizopodia of a more specialised type, with an axial rod surrounded by a thin layer of streaming cytoplasm, are seen in Cyrtophoraceae (Chromulinales, Pascher, 1911 b), a family of epiphytes in which a ring of such structures is produced around the front end; in *Cyrtophora* (Fig. 7 L) a well-developed cilium is present, but in *Palatinella* it is very small and insignificant, the degree of differentiation of the cilium being in inverse ratio to the perfection of the rhizopod-system.

Whilst in the cases just considered swimmers are still produced in the course of reproduction, a considerable number of permanently rhizopodial Chrysophyceae are known which have been grouped by Pascher (1913 a, p. 89) as Rhizochrysidales. A simple example of this type, showing many analogies with *Chrysamoeba* in its rhizopodial stage, is *Rhizochrysis* (Fig. 7 M) in which the individuals bear delicate, sometimes branched rhizopodia, but otherwise show all the typical characteristics of Chrysophyceae (cf. also Smith, 1920, p. 77). In Lauterborn's *Chrysidiastrium* (Pascher, 1913 a, p. 91) the individuals are united by coarse protoplasmic strands into short chains. We owe to Pascher (1915 a) the description of a number of striking epiphytes of this type—*Chrysocrinus*, the marine *Chrysothylakion*, and the remarkable *Rhizaster*. The latter shows resemblances to the Cyrtophoraceae, the stalked individuals contained within a close-fitting envelope with a wide aperture bearing a system of long horizontally extended rhizopodia grouped around the amoeboid front end. The epiphytic *Lagynion*, parallel with the encapsuled epiphytes mentioned on p. 117, bears but a single rhizopodium, whilst *Heterolagynion* (Pascher, 1912 b) is a colourless form of the same type betraying its affinities by the possession of leucosin.

The examples cited are sufficient to show that among Chrysophyceae (also in other classes according to Pascher<sup>1</sup>, although the relevant data are not at present available) we can trace a practically complete series of transitions to rhizopodial organisation. Commencing with forms like *Chrysamoeba*, where the latter is only a temporary phase, we pass to others (*Chrysopyxis*, Cyrtophoraceae), where the flagellate individual is produced only in relation to reproduction, and from these again to the permanently rhizopodial Rhizochrysidales where ciliate stages are unknown. A last step, involving the loss of chromatophores, would bring us to typical Rhizopods which, unless they retained some characteristic assimilatory product or other distinctive feature, would no longer be recognisably derivable from any pigmented group (cf. the colourless amoeboid forms mentioned above, p. 137).

Loss of chromatophores may take place in two different ways. In the first case the chromatophores do not divide at the same rate as the protoplasts, with the result

<sup>1</sup> Pascher (1917 a, p. 75) mentions that the swimmers of some Radiolarians are remarkably like naked Peridinieae.

that some of the individuals receive none of the former. This not uncommonly occurs in *Rhizochrysis* (cf. Fig. 7 *M*), but according to Pascher (1917 *a*, p. 43, where other instances are mentioned) such colourless individuals do not appear to possess much vitality. He is of the opinion that colourless forms more usually arise by progressive reduction of chromatophores in organisms that have acquired amoeboid or rhizopodial organisation and a consequent capacity for holozoic nutrition. He mentions (*ibid.* p. 47) a number of instances of rhizopodial Chrysophyceae with small pale-coloured chromatophores.

It need scarcely be pointed out that rhizopodial forms may also have been derived directly from colourless Flagellata (Pascher, 1917 *a*, p. 40). Thus, certain colourless forms are known which may be on the one hand either amoeboid or rhizopodial and, on the other hand, flagellate, being thus comparable to *Chrysamoeba* among pigmented types; some of these are classed among Rhizopods, others among Flagellata. *Actinomonas*, usually referred to Pantostomatinae, may altogether lose its cilium in the sedentary stage and be completely rhizopodial (Pascher, 1917 *a*, p. 71); this form occasionally produces leucosin and is probably a Chrysomonad.

#### 14. PLASMODIAL TENDENCIES AMONG PROTOPHYTA.

In certain of the rhizopodial Chrysophyceae the individuals cohere by coarse or fine protoplasmic strands to form chains (*Chrysidiastrium*) or nets (*Chrysarachnion*, Pascher, 1917 *a*, p. 54), and this may be regarded as a preliminary step towards the completely plasmodial condition realised in Pascher's *Myxochrysis* (Pascher, 1916). In the normal condition this organism occurs as a multinucleate plasmodium surrounded by a coarse envelope (Fig. 7 *G*), coloured brown by ferric hydroxide. Within the protoplasm there are usually numerous small plate-shaped chromatophores, although some plasmodia are colourless; there are numerous contractile vacuoles, oil-drops, and leucosin-masses. Movement is effected by blunt pseudopodia and the organism exhibits both holophytic and holozoic nutrition. Fusion of plasmodia commonly occurs. At times the protoplasm becomes cleft into numerous portions which are uni- or multi-nucleate, with or without chromatophores, and which subsequently encyst and develop a thick membrane (Fig. 7 *P*). From these cysts there arise, with or without previous division of the contents, *Chromulina*-like swimmers (Fig. 7 *N*, *O*) which may or may not possess a chromatophore and which after some time lose their cilium and become amoebae; sometimes, however, the swarming stage is suppressed and the amoebae are produced at once. The latter grow and their nuclei divide and sooner or later they form the characteristic envelope; but at all stages of their development fusions can take place and there thus result the large plasmodia that constitute the normal vegetative condition. It seems that sometimes the swimmers or amoebae may arise direct by division of the protoplasm of these plasmodia without the above-mentioned encystment occurring. The formation of colourless monads and from them of colourless plasmodia depends

on the fact that, in the division of the protoplasm, some of the products receive no chromatophores (cf. the case of *Rhizochrysis* mentioned on p. 140<sup>1</sup>).

Pascher (1918) has also described a species of *Chlamydomonas* (Fig. 7 R) with amoeboid gametes (Fig. 7 S), in which the amoeboid zygotes creep about on the substratum and fuse to form multinucleate plasmodia (Fig. 7 T), which ultimately encyst. Similar cases are mentioned for *Draparnaldia* (Pascher, 1918 a, p. 373). He sees in such behaviour an illustration of the possible method of evolution of the Myxogasteres among Myxomycetes (Pascher, 1918 a), where likewise the plasmodia originate as a result of sexual fusion and are therefore diploid.

#### 15. GENERAL CONCLUSIONS TO BE DRAWN FROM THE PREVIOUS CONSIDERATIONS.

The eleven classes of pigmented Protophyta that have been distinguished in the foregoing appear as so many separate physiological series, each possessing a different metabolism. In no two classes do chromatophore-pigments, reserve-substances, and cell-membranes show complete correspondence, although, as pointed out above (pp. 121, 134), there are considerable resemblances in various cases. On the other hand, in many classes, there are marked and pronounced differences in these respects, and these are accompanied by morphological distinctions of equal importance. In the writer's opinion it would be best to regard each of these classes as a perfectly separate evolutionary series, without any connection with the others, until such connection is indubitably established. It does not seem that this can be said for any of the existing evidence.

Attention may be drawn to the fact that, whereas in some classes (e.g. Heterokontae, Phaeophyceae) there is marked constancy in all the essential features that may be regarded as characterising the class from the point of view of a flagellate origin, in others one or more of the features are variable. This applies especially to the pigmentation in Cryptophyceae, Dinophyceae, Rhodophyceae, and Myxophyceae, to the ciliary numbers in Isokontae and Chrysophyceae, and to a lesser extent to the products of photosynthesis. Much more research will be necessary before we are able to estimate the significance of these variations, but in them may lie the key to a possible affinity between some of the classes. It seems unlikely, however, that many of the eleven classes of pigmented Protophyta here distinguished will be found to have a common origin.

Pascher, if I understand him rightly, is of the opinion that the colourless Flagellata will all prove to be reduced from pigmented groups (Pascher, 1914, p. 138; 1916 a, esp. p. 444, etc.) and evidently holds the same view as to the Fungi (1916 a, p. 445; 1917 a, pp. 8, 39, 76) and some of the Rhizopoda (1917 a, p. 76). As regards the Fungi there is, as indicated on p. 136, very little direct evidence to support such a view; and, whereas it has undoubtedly been shown that some colourless Flagellata are derived from pigmented types, this does not prove or even

<sup>1</sup> An analogous form may be *Chlamydomyxa* (Archer, 1875), which has also been investigated by Hieronymus (1898) and Penard (1904). Pascher (1925, p. 47) suggests it may be a member of Heterokontae.

render likely that all of them have originated in this way. We are not yet at least justified in doubting that colourless Flagellata may have arisen quite independently, and the same will apply to Rhizopoda. It is impossible to deny that colourless saprophytes and even holozoic forms might have originated simultaneously with holophytic forms.

This raises the question of the status of the present-day Protophyta. Are we to regard them as direct descendants through the ages of the original Protista, or are they, while repeating a simple construction, of more recent origin? It will be familiar that there are several instances of higher plants that, as the fossil records show, have apparently persisted with little alteration throughout long periods of the earth's history. This is true also of Algae; a *Cymopolia*, found in the Eocene sands of the Paris basin, is very little different from the *Cymopolia barbata* of the present day (Seward, 1898, p. 172), while some of the Tertiary and Cretaceous Diatoms are identical with existing species (West, 1916, p. 118). But, if such relatively specialised forms have persisted with little change, it is not improbable that the simplest aquatic Protophyta, for whom the conditions cannot appreciably have altered, have in part survived from earliest times. In the absence of all direct evidence on this point, caution is advisable, but it seems plausible to regard some of the simpler Protophyta of the present day as direct descendants of the early forms. But this is no doubt not true of all, nor need all the classes have originated at the same time. Organic life need not have started only in one epoch of the earth's history, and for all we know it may be arising at the present day. In groups like Isokontae and Chrysophyceae where, as above indicated, especially among the simpler forms, practically every conceivable variant of the central type appears to have been realised, many of the derived forms may well be of more recent origin than the central type. And it cannot be gainsaid but that classes like Chrysophyceae, with the vigorous many-sided development they show at the present day, may have a great future before them.

To the theory of the flagellate origin of the Algae there are now very few dissentients (cf. Mez, 1918, 1924; Steinecke, 1925), but the full consequences of the adoption of this view have been drawn by only very few. It has been shown in the preceding pages, and most clearly expounded by Pascher (esp. 1914), that, quite apart from the main algal classes and of the Heterokontae which are of more recent recognition, a number of classes of Flagellata (Chrysomonadales, Cryptomonadales, Peridinieae) have likewise evolved in the direction of the stationary plant and are now known to include forms that no one would hesitate to describe as Algae. In fact, it is only in Euglenineae and in the little-known Chloromonadales that, among holophytic Flagellata, such algal forms are still unknown. There is thus no further justification whatsoever for maintaining a group name Flagellata, since the organisms included in it belong to series that culminate in true chlorococcoid and filamentous Algae. To class forms like *Chromulina*, *Cryptomonas*, and *Gymnodinium* as Flagellata and *Chlamydomonas* as an Alga, as many botanists have done, is manifestly absurd in the light of our present knowledge. From this point of view it is most regrettable that, in the new edition of Engler and Prantl's *Natürliche*

*Pflanzenfamilien*, the old method of treatment is being maintained. The one volume published (Printz, 1927) deals with Isokontae and Heterokontae, which are more-over grouped together under Chlorophyceae, although at the present day all the evidence indicates that there is no question of any relationship between them. Evidently the "Flagellata" are to form a separate volume, and perhaps even the Dinophyceae will, as in the old edition, be divorced from this volume and treated of collaterally with the Diatoms. This practice is much to be deplored, especially in view of the example given by Oltmanns (1922). The view held by Chodat (1913, esp. p. 252 *et seq.*; 1925 a, p. 31), who groups all the classes above considered (except Isokontae) as Phaeophyceae, cannot be regarded as doing justice to our present knowledge of the forms in question. As pointed out above, it is inadvisable to accept relationships between the different classes, that are clearly marked out, until these can be based on overwhelming evidence.

At the same time I have no quarrel with the zoologist who chooses to include the flagellate members of the different classes as a group of Protozoa, as long as their plant characteristics are fully emphasised. They show many points of contact with unicellular animals and, although essentially holophytic in their nutrition, they are occasionally holozoic (p. 137). It would indeed be surprising if, in these simple forms, plant and animal characteristics were sharply segregated, but the pigmented Flagellata weigh down the scale on the plant side and, moreover, as has been seen, have mostly advanced very definitely in the direction of the sedentary plant. There can be little doubt that colourless classes of Protista exist independently of the pigmented ones, and it is to be presumed that some of these will have evolved into higher animal types.

Table showing parallelism in evolution of five classes of Protophyta. (In most cases more than the one instance cited under each category is known.)

Type of Construction	Isokontae	Heterokontae	Chrysophyceae	Cryptophyceae	Dinophyceae
(a) Motile holophytic unicell	Chlamydomonas, etc.	Heterochloris	Chromulina, etc.	Cryptomonas, etc.	Glenodinium, etc.
(b) Motile colourless unicell	Polytoma	Chloramoeba (facultative)	Oicomonas, etc.	Chilomonas	Oxyrrhis, etc.
(c) Encapsuled unicell	Coccomonas	—	Chrysococcus, etc.	—	—
(d) Motile colony	Pandorina, etc.	—	Synura, etc.	—	Polykrikos
(e) Dendroid colony	Prasinocladus	Mischococcus	Chrysodendron	—	—
(f) Palmelloid colony	Tetraspora	Chlorosaccus	Chrysocapsa	Phaeococcus	Gloeodinium
(g) Chlorococcoid (zoosporic)	Chlorococcum	Botrydiopsis	Chrysosphaera	Tetragonidium	Cystodinium
(h) Chlorococcoid (azoosporic)	Chlorella	Chlorobotrys	—	—	Hypnodinium
(i) Simple filament	Ulothrix	Tribonema	Nematochrysis	—	Dinotrix
(j) Heterotrichous filament	Stigeoclonium	—	—	—	Dinoclonium
(k) Prostrate filamentous type	Protoderma	—	Phaeodermatium	—	—
(l) Siphonous type	Codiaceae	Botrydium	—	—	—
(m) Holophytic amoeboid type	—	Rhizochloris	Chrysamoeba	Cryptochrysis amoeboides	—
(n) Holozoic amoeboid type	Gametamoeba	—	Leukochrysis	—	Dinamoebidium
(o) Plasmodial type	Chlamydomonas sp.	Chlamydomyxa?	Myxochrysis	—	—

A survey of the various classes of Protophyta, and especially of the pigmented ones which have been more particularly studied, shows a remarkable parallelism between them. Not only have different classes evolved the same main types of plant-body, as is illustrated in the above table, but in part these types are so similar in outward appearance that their apportionment to their respective



classes is only possible after investigation of the cell-contents and of the course of reproduction; in all probability many of the dubious forms of green Algae, etc., will on more careful investigation prove really to belong to other classes. It has been pointed out that in each class the different forms of body probably represent separate evolutionary lines, each a different attempt at the construction of a plant-body, but evolution seems to have followed broadly the same lines in every class.

In each of the main classes it is possible to recognise a definite upward tendency, accompanied by a greater and greater restriction of motility, and there appears to be no possibility of reading the sequence in the opposite direction. Church's view (1919, pp. 8, 46) that the simple freshwater Algae are starvation-forms reduced from more elaborate types lacks any evidence in its support; moreover, perfectly similar forms occur in the sea. The obvious parallelism between the different classes further renders such a view untenable. "We have no knowledge of any forms from which the filamentous Heterokontae or Chrysophyceae, for instance, could be derived by reduction....Until some real evidence can be adduced that reduction has occurred, it appears more logical to regard the filamentous forms in the different classes as the end-points of an upgrade development....Within the Isokontae anisogamy or oogamy are associated with the advanced forms and are mainly a feature of the specialised filamentous types, a fact which supports the idea of a progression, rather than of a retrogression. In other classes also sexuality is usually found only in those forms which have the more elaborate organisation" (Fritsch, 1927, p. 10). Again it is only in the highly organised Trentepohliaceae and Siphonales among Isokontae that special sporangia and gametangia are differentiated.

#### 16. THE RELATION OF THE PROTOPHYTA TO THE HIGHER PLANTS.

The most advanced type of simple filamentous body to be evolved is that which has above been called the heterotrichous filament. It is developed to a very high extent in the Chaetophorales (Isokontae), in the Ectocarpales, the Nemalionales, and evidently occurs also in Dinophyceae (p. 128) and not quite so clearly marked in Chrysophyceae; moreover, the Chamaesiphonales (Myxophyceae) appear to show the same construction. The heterotrichous filament is thus a stage reached in the evolution of many different classes, but, whereas in most it represents the culmination, in the two large marine classes, Phaeophyceae and Rhodophyceae, it constitutes the starting-point among the forms known to us at the present day. Moreover, it appears to be highly developed only in them and in the Isokontae, the only classes of Protophyta that are known to have attained to an oogamous sexual differentiation. It is well to note the contrasts: the Isokontae green and in their metabolism, etc., closely resembling land-plants, essentially freshwater, but with a very definite terrestrial tendency (Fritsch, 1921, p. 168), and terminating with forms possessing the heterotrichous filament and oogamy; the Phaeophyceae and Rhodophyceae with other pigmentation and metabolism, essentially confined to the sea, and, while beginning with forms having heterotrichous filaments,

attaining to a high degree of morphological and anatomical specialisation, although their reproductive equipment is not appreciably more advanced than that of Isokontae<sup>1</sup>.

Is it not probable that these facts have a definite interpretation? Why do the very vigorous Isokontae with their many-sided development leave off at a level at which the two marine classes begin? There is no reason why we should deny them a capacity for a further development, which brown and red Algae show us is possible from such a starting-point. The blind termination of so successful a group is probably indicative of a further evolution in some different habitat, for instance the land; and the absence of transitional forms is quite comprehensible, since such would probably succumb and only those capable of further adaptation persist. The evidence would seem to indicate that at about the evolutionary level exemplified by the heterotrichous filament the transition to a terrestrial existence took place, and that there are no higher Isokontae because they have become land-plants. Until the absence of more advanced green Algae has been explained, it is unwise to indulge in other speculative hypotheses having no foundation in fact.

The heterotrichous filament affords scope for the development of both prostrate and upright types (Fritsch, 1916), a form like *Draparnaldia* shows the beginnings of the differentiation of a main axis in the latter, whilst Ulvaceae (cf. also the terrestrial Prasiolaceae) exhibit an undifferentiated parenchymatous construction such as we find in the gametophytes of many simple terrestrial plants. We thus have all the different tendencies that appear necessary for the evolution of a simple green land-plant, and in this connection we must bear in mind the very marked capacity of many different Isokontae to exist under terrestrial conditions (Fritsch, 1922, p. 225), a capacity otherwise shared only by the blue-green Algae. That green Algae are capable of a more elaborate development is shown by the Siphonales which are essentially marine, but the types of construction evolved by them, like those of Phaeophyceae and Rhodophyceae<sup>2</sup>, have exceedingly little in common with the structure of any land-plant.

The oogamous members of Isokontae for the most part occupy a very isolated position and they appear as outliers, well in advance of the rest. The bulk of the filamentous green Algae are isogamous or anisogamous and, on the available evidence, there is no reason to suppose that the invaders of the land were further advanced in these respects. The oogamous members show the existence of the capacity for oogamy, but do not lie on the main evolutionary line.

The apparent absence of simpler Phaeophyceae and Rhodophyceae, than those which are included in Ectocarpales and Nemalionales respectively, seems to indicate that for them the conditions, that admitted of the development of the advanced types, were unfavourable and led to extinction. It is significant that these are the marine groups, for there has probably been more change in the sea than in fresh

<sup>1</sup> This is specially true of Phaeophyceae, while Rhodophyceae exhibit remarkable complexities in the events succeeding sexual fusion.

<sup>2</sup> In the vast majority, especially of the larger and more complex brown and red Algae, the body is built up in a way quite different from that of any land-plant.

waters. It appears to be generally accepted by geologists<sup>1</sup> that the first waters of the earth partook more of the nature of fresh than of salt waters. We may suppose that, as progressive increase in salinity of the oceans took place, some groups evolved in this environment, whilst others remained to populate fresh waters. Such an hypothesis would explain the fact that all classes have their marine and freshwater representatives, but that some are much more strongly developed in the one environment than in the other. It might also account for the absence of primitive brown and red Algae which might be supposed to have succumbed during the gradual concentration of the sea water.

Church (1919) has emphasised the many points of parallel between Phaeophyceae and Rhodophyceae and the higher land-plants, but in the writer's opinion these can only be taken as "a confirmation of the belief that environment has little to do with the broad evolution of the plant-organism and that these features are a natural outcome of the evolutionary trend in the Vegetable Kingdom and not any positive evidence for the view that they must necessarily have originated in a marine environment" (Fritsch, 1927, p. 12). The data afforded in the present article on the parallelism in the evolution of the different classes of Protophyta offer much support to the view of a general evolutionary trend.

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<sup>1</sup> Cf. e.g. Sollas, *The Age of the Earth*, p. 21. The biological facts seem to fall in line with such views.

<sup>2</sup> The papers cited have a direct bearing on the subject-matter of the present article. No attempt has been made to compile an exhaustive bibliography, even of recently described Protophyta, which would comprise several hundred references; for these, see Printz, 1927; West and Fritsch, 1927; Pascher, *Süßwasserflora* (1913 and onwards).

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# LIFE CYCLES IN THE PROTOZOA

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(Received 11 December, 1928.)

(With Ten Text-figures.)

THE intensive study of the Protozoa and the following out of their life histories constitutes a branch of biology which is of relatively recent date. The sequence of forms revealed and, as the technique developed, the nuclear changes accompanying them very greatly impressed the early workers, so that the idea of a definite life cycle came to bulk very largely in the views held about the Protozoa. Some of the sequences pieced together from incomplete or uncritical observation of living organisms and from the perusal of stained material, often of doubtful fixation, were quite fantastic. The technical obstacles to sound observation were very great and there was as yet no sufficient basis for criticism of the methods applied; moreover, the interpretation of the things seen presented serious difficulties. These last still remain in the investigation particularly of the more complex details of structure.

The present account deals with some of the more recent work on the life cycles of the Protozoa both in relation to the sequence of forms and the nuclear changes in the organism and to the environmental contact and stimulus.

Research into the life cycles has taken two main paths—in no sense actually opposed to each other, but nevertheless preoccupied with different aspects of the general data. The one trend has laid the greater weight of attention upon the sequence of morphological changes within the organism, the phenomena of sex, the details of nuclear behaviour and the comparison of these with one another and with the conditions found in metazoan cells; the other has been more interested in the behaviour of the organisms, their physiological activities and their reaction under experiment. The results needless to say have been mutually revealing in spite of individual divergences of outlook.

The antithesis expressed in these two main directions of investigation is a real one and we are faced with it in every life cycle investigated. There is a sequence of forms, but a more attentive enquiry shows that the passage from one stage to the next is the result of an internal state (often one particular internal condition) acted upon by an external stimulus. Klebs first formulated this fundamental principle that the cycle is dependent on (1) the inner self regulation of the organism and (2) the stimulus of the changing environment, in a series of botanical studies (Klebs, 1896, 1903, 1904 and 1910) that rank among the classics of biology. The conception has been elaborated since in the detailed observations and experiments of many workers. Some of these investigators are consciously working with a

recognition of Klebs' contribution; some, more particularly perhaps in America, are informed of the principle as part of the general stock-in-trade of modern biological ideas without apparently being aware of its origin; while some indeed seem unaware of the light it throws on the problems with which they are actually preoccupied. The cycles to be dealt with in the sequel will afford examples of Klebs' conception in nearly every instance where the sequence of forms has been more deeply studied. The organism cannot apparently carry out its cycle as an inevitable sequence entirely conditioned from within; in a perfectly stable environment which permitted of the life of the organism no cycle would exist (see *infra* Klebs, 1903, 1904; Calkins, 1926; Woodruff, 1925; Hartmann, Bělař, Beers, Dawson, etc.). But the *kind* of cycle is conditioned from within and belongs to a certain definite type. It can be interrupted and suppressed and there is often an element of opportunism in its evolution, but there is exactly the same general tenacity of individuality tempered by capacity for variation that is expressed in the more familiar forms of animal and plant life.

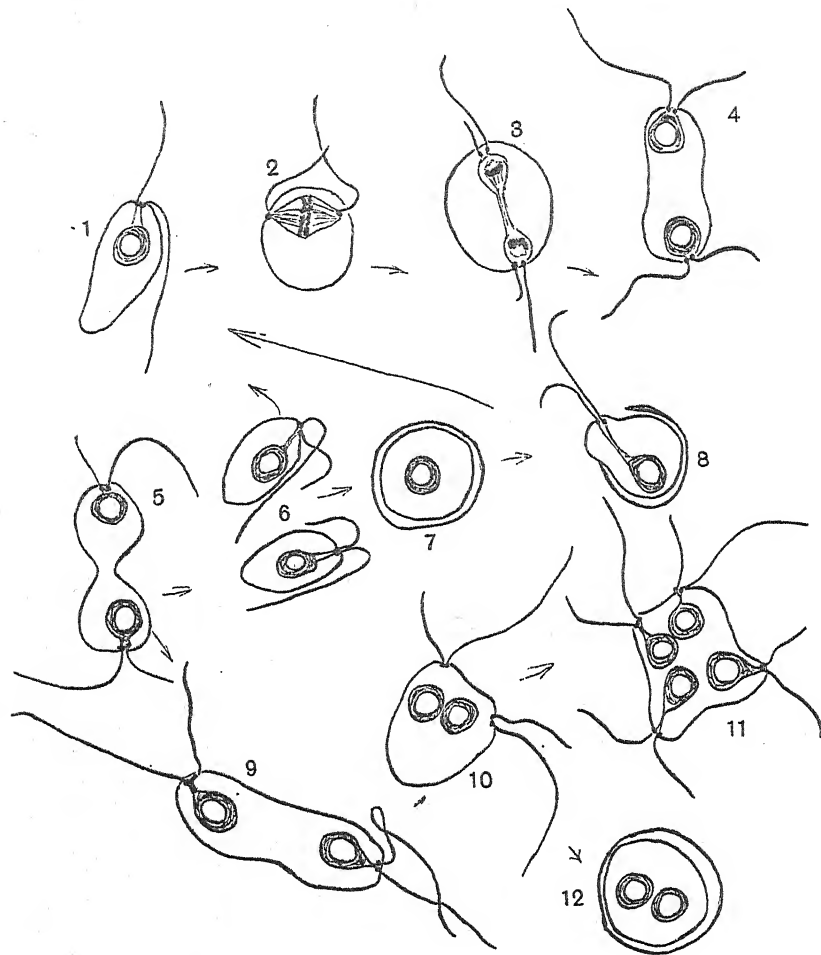
The life cycles considered here are taken in the order of the different classes—Flagellata, Rhizopoda, Ciliata and Sporozoa. Both simpler and more developed cycles are certainly found in each of the first three of these various groups, but on the whole this order gives a rising complexity.

The cycles in the free-living flagellates are among the simplest known in the phylum of the Protozoa. *Heteromita* (Robertson, 1928), Text-fig. 1, may be considered as an example, it is a small free living biflagellate organism. It has a vesicular nucleus with a central karyosome; the chromatin lies on or around the periphery of the karyosome and upon the inner side of the nuclear membrane. There are two unequal flagella which arise from two basal granules situated close together at the anterior end of the body. The basal granules are connected with the nuclear membrane by fine threads or rhizoplasts. *Heteromita* feeds upon bacteria and after a period of division the cultures encyst. Excystation can be provoked by placing the cysts in fresh medium or in distilled water. If the cysts are allowed to dry they can survive for at least one year and five months (Robertson, 1928); the cyst is here a protective structure, and no appreciable development takes place within it. It will become clear in the sequel that encystation is a phenomenon of the most varied significance and it must always be considered upon its individual merits in each life cycle. No one cause produces encystation and encystation is not the expression of any one internal state in Protozoa generally. It is curious that this obvious fact is not so generally appreciated as would be expected and there is still a certain tendency to look for a single cause of encystation (Kater and Burroughs, 1926).

In *Heteromita* the nuclear division is characterised by a well-developed mitotic spindle and the chromatin is arranged in an equatorial plate at the metaphase. The basal granules of the flagella play the part of centrosomes in the division of the nucleus. The normal division in the living state occupies from 7 to 15 minutes. If, after the division of the nucleus and the drawing apart of the main bulk of the protoplasm, there is any delay in the actual breaking through of the narrow junction



between the two daughter individuals, the periplast or external layer of protoplasm resumes a consistency that precludes protoplasmic division and a double individual equipped with two nuclei and two flagellar pairs with their basal granules sets out upon the interdivisional period of nutrition, growth and ordinary activity. In the



Text-fig. 1. *Heteromita*. (Diagram arranged from Robertson, 1928.) 1 to 6. Cycle of division. 7. Encysted individual. 8. Encysting individual. 9. A double individual formed as the result of an incomplete division. 10 and 12. Encystation of a double individual. 11. Division of a double individual into four.

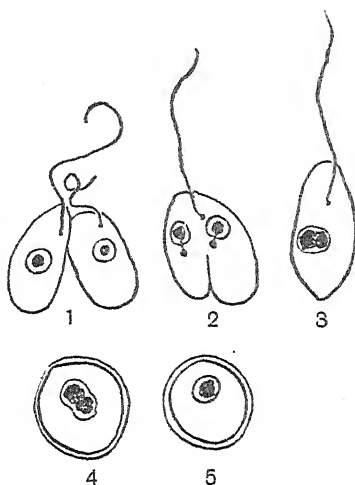
course of a few hours, usually 3 to 6, a second division occurs and each nucleus undergoes mitosis and the creature splits into four daughter flagellates. The explanation seems to be that the division of the protoplasm appears here to be a function of the telophase, that is, of what may be called the downward or return grade of the mitotic process. T. B. Robertson (1909) suggested that the equatorial lowering of surface tension and consequent streaming phenomena which lead to

cell division are brought about by cholin or soaps of cholin liberated in the cell through the splitting of lecithin in nuclein synthesis.

It may be added that these doubles may encyst but the nuclei do not fuse, and after resting for weeks or months will come out again as binucleate creatures. The interesting thing here is that these are not stages in any form of syngamy or autogamy. The writer has observed exactly the same phenomenon in *Cercomonas*—another biflagellate semi-amoeboid organism. A similar formation of double individuals arising from an incomplete or delayed division has been observed in *Noctiluca* by Pratje (1924).

In the flagellates the cycles seldom show syngamy. Three well authenticated instances of the process have however been described. An isogamous fusion of gametes takes place in *Scytomonas pusilla*, Text-fig. 2 (Dobell, 1908) in *Helkesimastix faecicola* (Woodcock and Lapage, 1915) and in *Polytoma uvella* (Krasilschtski, 1882; Wenyon, 1926). In *Polytoma uvella* the process can be set off with the greatest regularity by confining well-fed individuals in a watch-glass or in a hanging drop (unpublished observations of the writer). In these cases there is no obvious sexual differentiation of the gametes. The question of sex in its relation to syngamy is discussed in a later part of the paper. It is important to have established the fact of syngamy among the flagellates, for in the great majority of the group very careful study has failed to reveal the process.

The absence of syngamy seems to have an interesting bearing on the character of certain flagellate genera. In the very widely distributed genus *Trypanosoma* in which syngamy has never been correctly observed, there is shown a well-developed capacity for adaptation to the circumstances of di-genetic parasitism. This high degree of adaptability is correlated with the remarkably labile character of the trypanosomes and the species are in many cases ill defined. Artificial drug-fast strains can be produced in the laboratory and natural strains of distinct character are constantly being found by workers in the field in Africa. So variable are the strains that each one studied has to be considered upon its own merits in regard to a great number of its biological characters. Moreover, the nature of individual strains subject to the active reactions of the blood of the vertebrate host is constantly changing, particularly in the disease-producing types. Without entering into the very interesting question of the mechanism by which these altered strains are produced, there is clearly, both in the drug-fast and modified passage strains and in the variety in the character of natural strains, an example of the accumulation



Text-fig. 2. Diagram of copulation of isogametes of *Scytomonas pusilla*. (After Dobell, 1908.) 1. Union of isogametes. 2. Reduction of the nuclei. 3. Fusion of the nuclei. 4 and 5. Encystation of the zygote.

of a heritable modification in character. A natural example is the loss of transmissibility by Glossina of *T. gambiense* reported by Duke (1927) and a similar condition of variety in strain in regard to transmission in the laboratory is described in the nearly allied genus *Leishmania* by Hindle (1928).

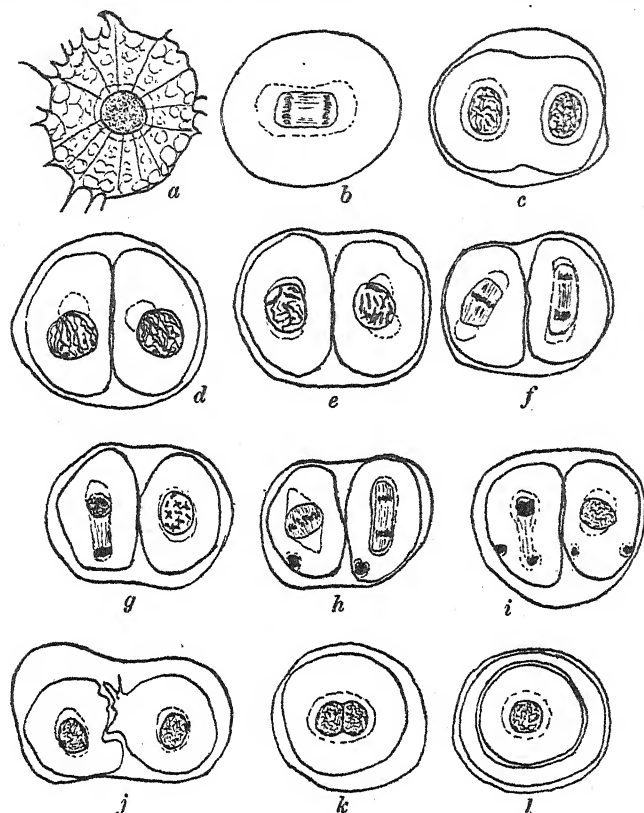
Jollos (1921) obtained heritable modifications in *Paramecia* strains subjected to the action of arsenic and the key to the understanding of the process was afforded by the return to the normal in the great majority of the strains upon nuclear reorganisation in endomixis or conjugation. There is no question of mutation in this type of change in the trypanosomes (and indeed there is no real means of testing a true mutant in such a genus). It appears to be a matter of heritable modifications impressed upon the strains by the environment and uncorrected by a reorganisation process. That these heritable modifications in drug-fast trypanosomes are very deeply impressed upon the strain has been shown recently by the character being retained after cyclical transmission by means of tsetse fly in *T. gambiense* (Duke, 1927) and by the flea in *T. lewisi* (Reichenow and Regendanz, 1927). The labile character of trypanosome species seems to have been on the whole of advantage to the genus, as it is extremely widely distributed and there is hardly a vertebrate group which does not harbour an example.

The class of the Rhizopoda reveals a relatively very small number of completely worked-out cycles, and some of the cycles formerly accepted are now considered to be erroneous.

In *Actinophrys*, recently described in great detail by Bělař (1924), the life cycle proceeds by binary fission and there is a very well-developed nuclear mitosis. A paedogamous conjugation occurs of gametes derived from the immediately preceding division of one single individual, Text-fig. 3. There are two maturation divisions of the nuclei in each partner. The first is the reduction division whereby the diploid number of chromosomes is reduced to half. The two polar body nuclei in each individual degenerate. At this stage Bělař describes an increased activity in one of the gametes and the extrusion of a pseudopodium. This more active partner to which he attributes a certain degree of maleness moves towards the passive one and unites with it, the nuclei fusing. The zygote secretes a cyst which upon being removed into a fluid of lesser osmotic pressure germinates and the *Actinophrys* proceeds upon its active life again. Bělař found that conjugation could be induced or prevented according to the adjustment of the conditions of culture and also that under uniformly favourable conditions *Actinophrys* could be cultivated indefinitely (actually for  $2\frac{1}{2}$  years and to 1244 generations) without any conjugation, or any other form of nuclear reorganisation, beyond that of normal mitotic division. That is to say that this very definite sexual cycle was shown to be dependent upon an external stimulus from the environment, and further it could be suppressed indefinitely without loss of vigour if the surrounding conditions were stabilised and kept uniformly favourable.

Paedogamy is a curious phenomenon originally described in the cysts of *Actinosphaerium* by Hertwig (1898). It is a very special case of syngamy and as manifested in *Actinophrys* is a self-regulatory phenomenon of great interest. The

physiologically distinct sexes of the equal gametes is particularly interesting in an organism of this type and, as we shall see later, there are excellent cases of physiologically distinct isogametes in *Gonium* and *Pandorina* which are haploid organisms. Bělař accepts the bisexual theory of the nuclear make-up and considers that this is an example of "relative sexuality," one member discarding the more female portion of its chromatin during maturation, the other discarding the more



Text-fig. 3. Diagram of the paedogamous copulation of *Actinophrys sol*. (After Bělař, 1923.) *a*. Withdrawal of pseudopodia. *b, c*. Production of the gametes by division of one individual. *d, e*. Preparation for the first maturation division which is the reduction division. *e*. Shows the strepsinema stage with the split and twisted chromosomes. *f*. First maturation division. *g*. Formation of the first polar body. *h*. Second maturation division. *i*. Formation of the second polar body. *j*. Copulation of ripe gametes. *k*. Zygote with the nuclei about to fuse. *l*. Zygote cyst.

male portion. The theory of relative sexuality and its bearing on syngamy is discussed in a later part of the paper.

A great deal of interesting work has been done upon the life cycles of the ciliates; they have afforded material for much varied and delicate experimental work which has yielded a considerable body of knowledge. The ciliates are characterised by a very great capacity for movement and have in many cases developed complicated organelles and appendages. In this group also the nuclear functions

have been divided into two departments, the generative and the vegetative, and this is expressed morphologically by the division of the nuclear apparatus into a micronucleus and a macronucleus. The activity of the body and its general character is so highly developed that there was probably a call for a large amount of vegetative chromatin to carry out the physiological function, whatever its nature may be, that is effected by this substance.

The macronucleus is derived from the micronucleus, it degenerates and is reformed anew at certain periods. Biologists, it may be remarked, are on firmer ground than is often the case in attributing a vegetative character to the macronucleus, for amiconucleate races have arisen in which only the macronucleus is present and all the vegetative functions including binary fission can go on indefinitely, but conjugation cannot be carried out (Patten, 1921; Woodruff, 1921; Dawson, 1919).

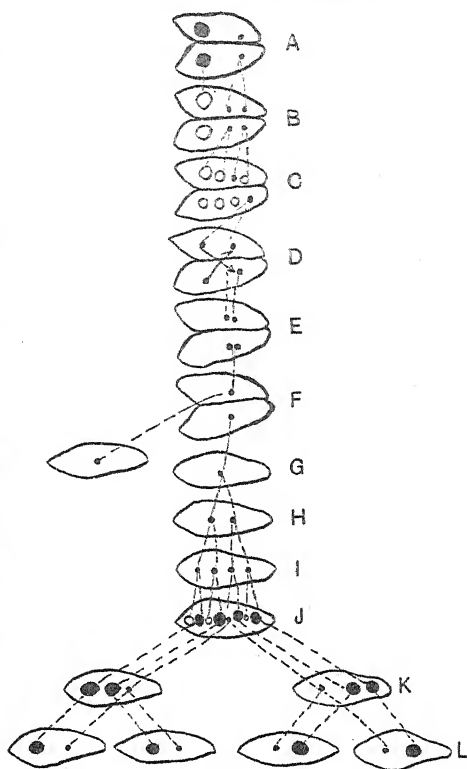
Moore (1924) has however questioned the interpretation of the nuclear condition of these races as being wholly macronuclear in type. In regeneration experiments she comes to the conclusion that some idiochromatin (micronuclear generative chromatin) is necessary for normal asexual reproduction. And she considers that it is highly probable that the nuclear equipment of the amiconucleate races represents an amphinucleus which has arisen through some irregularity during conjugation. As the macronucleus derives from the micronucleus it seems not impossible that it might in amiconucleate races have a composite character and retain both elements.

The life cycles of most ciliates are externally relatively simple; division is binary and transverse and there is a division (or in many forms a reconstruction) of the chief organelles. The micronucleus divides by mitosis (which is, in passing, an added argument for the importance of an equal division of chromatin in the nucleus that is involved in inheritance) while the macronucleus simply draws out into two approximately equal portions which separate. Encystation occurs and as always the device may be used for various purposes. The organism may encyst for protection as happens at some time or other in most ciliates even in *Paramecium* (Michelson, 1928), for division as in *Colpidium cucullus* (Wenyon, 1926), for conjugation or to carry out nuclear reorganisation as in *Didinium nasutum*, *Prorodon griseus*, *Spathidium spathula* and *Chilodon* (Mast and Ibara, 1923; Tannreuther, 1926; Moore, 1924; Ivanić, 1928). Encystation does not occur casually but is a response to external conditions, though the stimulus to encystation does not seem to be the same in different instances (Beers, 1927; Brand, 1924; Manwell, 1928; Stolte, 1924; Michelson, 1928).

A typical cycle under "wild" conditions in such a ciliate as one of the *Paramecium* species is as follows. Food is ingested and growth and maintenance is carried out under conditions of great activity of movement. Divisions take place at regular intervals, usually of about 24 hours or less according to conditions. At certain periods epidemics of conjugation occur. The following is a very brief general description of the process which consists essentially in an interchange of nuclei, each partner then separating while the received nucleus fuses with the stationary



nucleus (Text-fig. 4). The two conjugants come together and are apposed; the protoplasm, usually towards the anterior end in the region of the cytostome, fuses together so that there is a continuous protoplasmic bridge between the two ciliates. The micronuclei in each individual divide twice in succession producing four. One of these divisions is a reduction division. In *Chilodon unciatus* (MacDougall, 1925) it is the second, as it is also in *Prorodon griseus* (Tannreuther, 1926) and in *Pleurotricha lanceolata* (Manwell, 1928). Of the four nuclei in each conjugant three degenerate, while the remaining one divides into two. These are now known as the pronuclei. One of them in each case remains stationary and the other wanders over into the opposite partner and unites with the stationary nucleus, the fused nuclei adopting the spindle shape. Each of the conjugants has one micronucleus, the synkaryon, the macronucleus in process of degeneration, and the degenerated micronuclei rejected at the maturation divisions. The conjugants can now be called zygotes. They separate and the reorganisation begins in each. By three successive divisions eight micronuclei are formed, three of which degenerate. Of the remaining five one becomes the permanent micronucleus and the other four give rise to the four macronuclei of the four individuals produced in the next two divisions of the *Paramecium*. This process of conjugation is entirely different from anything occurring in the other groups. It is not always carried out between equal conjugants and in the case of *Vorticella* (Maupas, 1888) there is developed an actual microgamete in the shape of a small vorticella which attaches itself to the macrogamete. The nuclear story goes on as above, but after the production of the synkaryon or zygote nucleus in the macrogamete the microgamete degenerates and dies. Another modification has been described by Noland (1927) in *Metopus sigmoides*, where the pronuclei from



Text-fig. 4. Diagram showing the nuclear changes during conjugation of *Paramecium caudatum*. (After Jennings, 1920.) A. Two associated conjugants. B. Degeneration of macronucleus and first division of micronucleus. C. Second division of micronuclei to give rise to four, of which three degenerate. D. Division of remaining micronuclei to produce the gamete nuclei. E-F. Union of gamete nuclei. G. Separation of the conjugants. H-I. Division of the nuclei to give rise to eight, of which four increase in size to become macronuclei, while three degenerate. K. After division of the single micronucleus the ciliate itself divides. L. After a further division of the micronucleus the daughter ciliates again divide to give rise to the normal type.

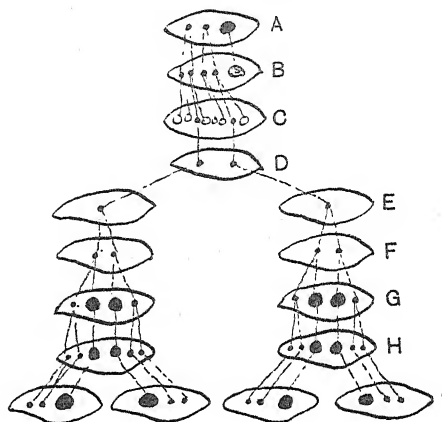
on as above, but after the production of the synkaryon or zygote nucleus in the macrogamete the microgamete degenerates and dies. Another modification has been described by Noland (1927) in *Metopus sigmoides*, where the pronuclei from

the smaller conjugant flow with a large portion of the cytoplasm into the body of the larger partner. The nuclear sequence is much as in the type already described but here too as in *Vorticella* the smaller gamete is sacrificed.

Another important nuclear process called endomixis (first fully worked out by Woodruff and Erdmann, 1914) must be described briefly before the importance of the nuclear behaviour in relation to the life cycle and multiplication is discussed (Text-fig. 5).

At periods of a few weeks in flourishing cultures of *Paramecium aurelia* a process of nuclear regeneration takes place whereby the macronucleus degenerates. The micronuclei (there are normally two present in this species) undergo two successive divisions producing eight micronuclei, of which either six or seven degenerate. If six are absorbed a division of the whole ciliate produces individuals each with one micronucleus and no macronucleus. In either case there is now an animal with a single micronucleus. Reorganisation takes place exactly upon the lines that have already been given in the case of the exconjugants described above, resulting in the formation of a new macronucleus from one of the micronuclei. The physiological advantage of a new macronucleus is probably very considerable: the advantage to the micronucleus is not so obvious, but the process is not completely known. The very important detail that still requires further investigation is whether there is any sign of nuclear reduction at the first or second division of the micronucleus. There is a general consensus of opinion that fusion of the micronuclei does not occur in endomixis; that is to say that it is not an autogamous conjugation. This reorganisation is called endomixis, but this is an unsuitable name; it is really a form of parthenogenesis.

When endomixis was first discovered in *P. aurelia* by Woodruff and Erdmann (1914) it was expected that it would be found to be a generally distributed phenomenon throughout the Ciliata. This has not proved to be the case; it is known in *P. aurelia* (Woodruff and Erdmann, 1914), in *P. caudatum* (Erdmann and Woodruff, 1916) and in *P. polycaryum* (Woodruff and Spencer, 1923) in the free swimming state and it also occurs in *Spathidium spathula* (Moore, 1924) and *Didinium nasutum*, *Uroleptus* and *Chilodon* in the encysted state. Curiously enough it does not occur in *P. calkinsi* (Spencer, 1924). Endomixis is an interesting self-regulatory process



Text-fig. 5. Diagram showing nuclear changes in *P. aurelia* during endomixis as described by Woodruff and Erdmann 1914. (After Jennings, 1920.) A-B. Degeneration of macronucleus and first division of the two micronuclei. C-D. Second division of the micronuclei and degeneration of six of the daughter micronuclei. E. Division of the ciliate to produce two daughter individuals each with a single micronucleus. F-G. Two divisions of micronuclei to give rise to four, two of which increase in size to become macronuclei. H. Further division of micronuclei. I. Division of ciliate to give rise to the normal type as at A.

but it does not seem to be one that is essential to ciliates generally. Calkins and Bowling (1928) suggest a derivation of endomixis from a paedogamous copulation which they find in *Dallasia frontata*.

It was said above that after a period of multiplication conjugation took place and many workers (Bütschli, 1876; Englemann, 1876; Maupas, 1889; Hertwig, 1900; Calkins, 1902 and 1904) came to the conclusion that cultures died out if the sexual interchange did not take place and from this they drew the deduction that conjugation was a process of rejuvenescence. This alternation of periods of division and epidemics of conjugation was called the life cycle and an extensive structure of theory was built up on the basis of these observations. More careful control of the medium showed that *P. aurelia* and *P. caudatum* could be cultivated for hundreds of generations (6000 and more) under uniformly favourable conditions without the appearance of conjugation (Woodruff, 1920; Calkins, 1926), and that the rhythmic fluctuations in the rate of division which occurred every three or four weeks corresponded with periods of endomixis. These rhythms in division rate were afterwards found also in a species of *Paramecium* (*P. calkinsi*, Spencer, 1924) and in *Spathidium spathula* (Moody, 1921; Patten, 1921) without the occurrence of endomixis.

In 1926 Unger carried out an interesting and detailed investigation with three species of *Paramecium*, *P. aurelia*, *P. calkinsi* and *P. caudatum*. He found that at the tip of the wave of the fission rate rhythm there are more food vacuoles formed than at the bottom and that the contractile vacuole pulsations are more active. He concluded that "rhythms in the division rate and fluctuations in the metabolic activity of *Paramecia* are not intrinsically the result of nuclear reorganisation processes, temperature variations or differences of food material, but of some unknown, intrinsic physiological factor in the protoplasm of the cell."

This deduction, however, does not seem to the present writer to be established, as Unger has not investigated either the stimulus to ciliary movement, the causation of food vacuoles, or the stimulus to pulsation in the contractile vacuoles in relation to the environment at the two periods. His apparently stable environment is open to variations that escape his methods of detection and the value of the bacteria as food and energy producers at the two periods is assumed to be similar. An important point in Unger's paper is that the rhythms of reproductive activity are present in *P. calkinsi*, which does not undergo endomixis, as well as in *P. aurelia* and *P. caudatum* which do. Two papers of Beers (1928, 1, 2) throw a light on these rhythms which suggests that they, like conjugation, are produced by external stimuli from the environment. He works with *Didinium nasutum* where the conditions of culture could be very carefully standardised. It feeds not upon bacteria but upon *Paramecia*, and it also undergoes endomixis in the encysted state only. Beers made up a synthetic spring water of standard composition and fed the *Didinium* upon flourishing and healthy *Paramecia* and found that the rhythms in the reproduction rate disappeared. He then found he could induce artificial reproductive rhythms by feeding starved *Paramecia* to the *Didinium* thus lowering the fission rate which reached the normal figure again when the diet of healthy

*Paramecia* was resumed. He also (1928, 1) produced abnormal and senescent strains which finally died out by feeding *Didinium* continuously upon starved *Paramecia*. The experiments appear to be carefully carried out and the results are of great interest. Related to this also is the work of Dawson (1928) who shows that "trends" in long continued cultures are the same under parallel conditions in various widely separated genera. He worked with *Histrio complanata* (order *Hypotricha*), mutant *P. aurelia* (order *Holotricha*) and *Blepharisma undulans* (order *Heterotricha*). The "trends" of fission rate during four years' cultivation without the occurrence of either endomixis or conjugation under identical conditions were similar in all three genera, and he pointed out that the "trend" need not always be downwards. This is also shown by Metalnikow (1922) who found the "trend" of his *P. caudatum* line cultures was upwards, *i.e.* the general curve of division rate was rising after ten years' cultivation without conjugation.

The distinction between the fission rates of lines selected from the anterior of the two daughter ciliates produced at each transverse division has been investigated by de Garis (1928), who brings evidence to show that heritable diversities can be produced by selecting cells of anterior or posterior origin. The fission rate may be higher in those of anterior or posterior origin, or (in a single observed case) they may be equal, but the direction of change is heritable so that if the increment is associated with a particular stock, for instance the anterior, it remains so associated. Child and Deviney's (1926) paper on the physiological gradient of *P. caudatum* has a bearing upon this work of de Garis. They found evidence for an axial gradient from before backwards. In *P. caudatum* they ascertained that the anterior vacuole beats usually more rapidly than the posterior but that this may be reversed under conditions of intoxication. Unger (1926), in *P. caudatum*, also found that the anterior vacuole beats generally more rapidly; but in *P. aurelia* it is the posterior one that has the more rapid pulsation and in *P. calkinsi* they are about equal. Taking the more rapid pulsation to indicate a more active metabolic rate he considered that there was a physiological polarity which is different in the three species. In no species is the physiological polarity absolute and a low percentage of individuals with reversed or equal polarity are always to be found. Unger is here in opposition to Child and Deviney who considered that the antero-posterior gradient is primarily a quantitative differential including both metabolic and physical factors and that "there is no evidence for the existence of any other basis of physiological polarity than this gradient." The work of de Garis however supports Unger's view rather than Child and Deviney's.

In Unger's paper lies also the explanation of Parker's (1927) results. Parker makes a rapid and a slow selection in regard to division rate, and succeeds in *P. aurelia* and in *Stylonychia* in producing a slow and a fast heritable line. He fails to do this with *P. calkinsi*. He finds a very interesting fact that the number of selections has no clear relation to the effect produced. This is no cause for surprise if the character selected is really a heritable quality. Garis also found this. Parker concludes, with insufficient evidence, that the effectiveness of selection depends on endomixis. Endomixis occurs in *P. aurelia* and does not in *Stylonychia*, so he

assumes that an invisible endomitic process must take place in *Stylonychia*. In *P. calkinsi* the clone could not be selected into two heritable lines and he assumes that this must be owing to the absence of endomixis which, as is well known, does not occur in this species. He neglects Unger's work showing that in *P. aurelia* there is a physiological polarity and that the contractile vacuole of one end (in this case the anterior) beats more rapidly than the other, which enabled him to divide the clone into a more rapid fission line and a less rapid fission line upon the difference in metabolic activity of the two ends of the single clone parent. In *P. calkinsi* there is no such marked physiological "polarity" and the two vacuoles beat at the same rate. It is much more probable that it is this and not the absence of endomixis that accounts for his inability to divide the clone upon this particular quality of rapidity of fission rate.

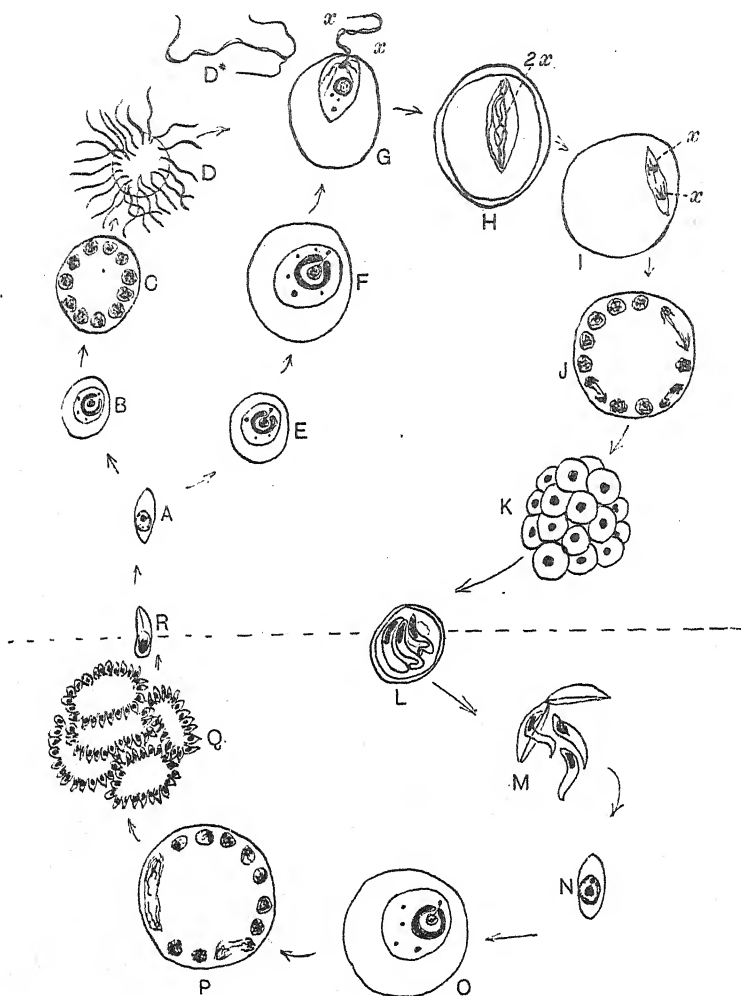
As the outcome of all the experimental work discussed above, the American workers deny the existence of a life cycle in ciliates. To the present writer this seems an erroneous attitude. The cycle can be induced at any time by altering the conditions, and the appearance of the cyclical processes can be correlated with changes of the environment brought about by the metabolic products of the organisms themselves or other appreciable changes (Bělař, 1924, 2; Austin, 1927, etc.). Chatton, E. and Chatton, M. (1923, 1, 2 and 1925) obtained a very delicate control of the ciliate *Glaucoma scintillans* and were able to induce conjugation by altering the conditions of nutrition and the composition of the medium. The reduction of the concentration of the salts (within certain limits) in proportion to the amount of sugar present inaugurated an epidemic of conjugation with the greatest regularity. The result of this series of investigations is to show that the cycle is not a rigid internally conditioned sequence but is the response of an internally adaptable organism to the external stimulus of the environment. What does seem to be inevitable is the exact nature of the response.

In approaching the Sporozoa we are confronted with the most highly developed and most definite life cycles to be found in the whole phylum. The recent work has produced detailed information especially concerning the reduction phenomena and fertilisation. Some of the important forms will be dealt with very briefly.

The coccidian *Aggregata eberthi* studied by Leger and Duboscq in 1908 has been the subject of a most detailed investigation by Dobell (1925). The sporozoites are liberated in the gut of the scavenging crab *Portunus depurator* (Text-fig. 6), and creep into the subepithelial connective tissue where they develop into schizonts. The adult schizont nucleus contains two bodies; a karyosome of nucleolar character and a chromatin body which Dobell calls the "micronucleus." The "micronucleus" enters into the enlarged karyosome by means of a minute pore or micropyle. At the first nuclear division the chromatin passes through the micropyle of the hollow karyosome and forms six very characteristic chromosomes. Dobell sees in the "micronucleus" the generative chromatin and the origin of the chromosomes. This account of the nuclear behaviour is criticised by Bělař (1926, 2) who considers that the chromosomes may be present along with the "micronucleus," and he doubts if Dobell's interpretation of the "micronucleus" as a nucleus is



correct. Dobell takes the view that the small nucleus is a generative micronucleus placed within a vegetative macronucleus comparable to that of the ciliates. Naville (1927, 2) also disagrees with this interpretation, he considers that the structure



Text-fig. 6. Diagram of the life cycle of *Aggrega eberthi*. (After Dobell, 1925.) The stages above the dotted line occur in *Sepia officinalis*, those below in *Portunus*. R. and A. Merozoites in *Sepia*. B, C, D and D\*. Development of microgametes. E and F. Development of macrogamete. G. Fertilisation. H. Zygote. G and H. Diploid stages. I. Sporont—reduction to haploid number of chromosomes occurs at the first nuclear mitosis. J. Sporont. K. Sporoblasts. L. Spore with three sporozoites. M. Sporozoites freed in intestine of *Portunus*. N-Q. Schizogony.

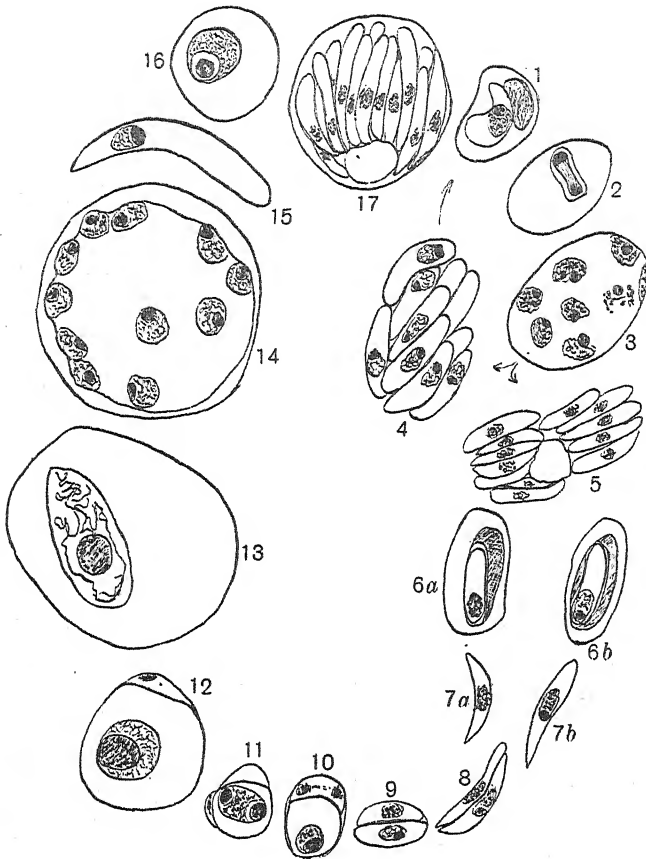
called the micronucleus contains chromatin but is not a complete nucleus, nor is there evidence for assuming that it contains all the chromatin of the chromosomes. The schizogony takes place in the connective tissue surrounding the gut of the crab and no further development occurs until the crab is eaten by the cuttle fish.

Then the merozoites penetrate into the intestinal wall of the latter and grow into large macro- and microgametocytes. The nucleus of the microgametocyte divides repeatedly and slender motile bi-flagellate microgametes are produced—the macrogametocyte grows into the single large egg-cell with one nucleus. The microgamete fertilizes this cell and the zygote is formed; the nucleus of each gamete containing six chromosomes. In Dobell's own words "the chromatin of the two gametes becomes inextricably commingled in the zygote nucleus shortly after fertilisation. From this mixed chromatin the peculiar 'fertilisation spindle' figure is formed, consisting of an elongate net-like arrangement of threads having no regular pattern. The number of the threads varies and there is no justification for calling them 'chromosomes.' The 'spindle' figure gives way to that which I have called the 'cobweb' figure, in which there is no trace of chromosomes, the chromatin being finely divided once more. From the 'cobweb' a spireme is formed, consisting of a single thread, not a double (diplotaene) thread." There are then formed 12 filamentar chromosomes consisting of six homologous pairs which are paired *a* with *a* and *b* with *b* as they take up their position on the equatorial plate. The mitosis proceeds and the pairs pull apart. Thus at the very first nuclear division within the zygote the number of chromosomes is reduced from 12 to 6, the diploid condition having lasted only during the short period of the association on the fertilisation spindle. The zygote nucleus divides a number of times and sporoblasts are formed by the segregation of the protoplasm around the nuclei. Each sporoblast secretes a cyst, the sporocyst, and three sporozoites and a residual body are formed within it. These cysts pass out to the exterior and are eaten by a crab and the cycle starts again.

In *Aggregata* there is a haploid cycle which is a condition found generally in the *Coccidia*. It is clear from Dobell's account that he cannot trace the chromosomes as persistent individuals during the stages which follow immediately upon the fertilisation (fertilisation spindle and "cobweb" stage). The six very distinct and easily identifiable chromosomes appear however in the very next stage and, before reduction, go through a conjugation closely analogous to that found in meiosis in the Metazoa. To the present writer it seems that too much weight should not be laid upon the apparent disintegration of the chromosomes in the fertilisation spindle. In the first place the difficulties of investigation are very great in a form of this type and it must also be borne in mind that, in addition to the possible errors of fixation, it is becoming increasingly clear that all the nuclear stains give a very poor account of chromatin in the interphases between the mitoses in the nuclei of the Protozoa. The actual disintegration of the chromosomes can hardly be held to be established on Dobell's data. It is indeed of greater interest that the six chromosomes should be so characteristic in shape and should reappear so invariably at every mitosis. It is also not manifest that the morphologically indistinguishable schizonts which give rise to male and female gametes are really of undifferentiated make up. This matter is considered along with other data in the sequel.

Another type of cycle which shows an adaptation to a life spent partly in

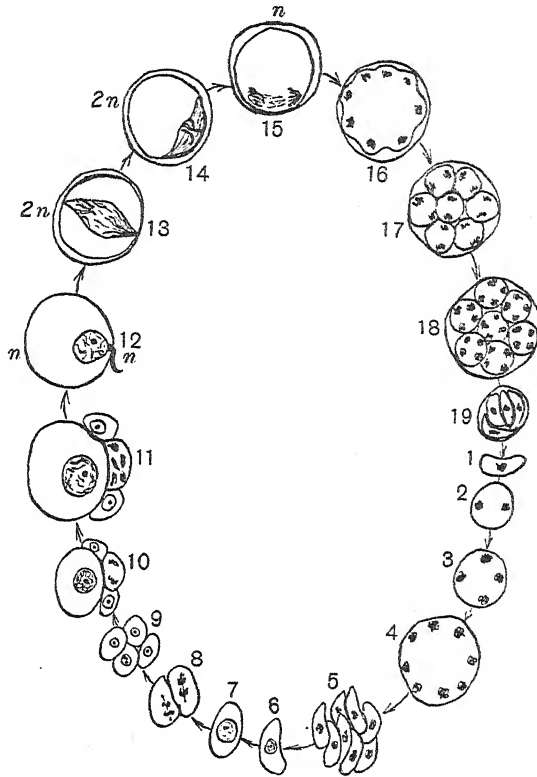
the blood stream of a vertebrate is shown in *Karyolysus lacertae* (Text-fig. 7) (Reichenow, 1921). *Karyolysus* is found as a haemogregarine in the blood corpuscles of the lizard; the parasites penetrate into an epithelial cell of the capillaries and schizogony takes place. The merozoites grow and repeat the schizogony, and



Text-fig. 7. Diagram of the life cycle of *Karyolysus lacertae*. (After Reichenow, 1921.) 1. Merozoite in the endothelial cell of a blood vessel of the lizard. 2-4. Schizogony. 5. Smaller merozoites formed later in the period of schizogony. These penetrate into the blood corpuscles. 6 a and 6 b. Male and female gametocytes. 7 a and 7 b. Gametocytes liberated from the blood corpuscles after being ingested by the tick *Liponyssus saurorum*. 8. Early association of the immature gametocytes. 9. The gametocytes creep together into a cell of the intestinal wall. 10. The macrogamete begins to increase in size. The microgametocyte divides into two microgametes. 11 and 12. Fertilisation, one microgamete penetrates into the macrogamete. 13. Fertilisation spindle. 14. Division of the zygote into motile sporokinetes which penetrate into the eggs of the tick. 15. Sporokinete. 16. Sporoblast derived from the sporokinete. 17. Sporocyst in the egg of the tick containing the sporozoites.

finally rather smaller merozoites are produced which penetrate into the blood corpuscles and are the future gametocytes. The blood is drawn into the tick *Liponyssus saurorum* and the gametocytes are liberated in the gut. An interesting process occurs at this stage, a slight differentiation into male and female is already present and the micro- and macrogametocytes now associate and become apposed

before maturation. They penetrate together into a cell of the intestinal wall and the maturation process proceeds. The macrogametocyte swells up to form a large round cell and the microgametocyte, which increases very little in size, divides into two microgametes one of which fuses with the egg cell and the other degenerates. The zygote grows very big and there is a fertilisation spindle as in *Adelea* and other Coccidia; the chromosomes conjugate and reduction takes place at the first mitosis of the synkaryon. There is here again a haploid organism with reduction within



Text-fig. 8. Diagram of the life cycle of *Klossia helicina*. (After Naville, 1927.) 1. Sporozoite. 2-6. Schizogony. 6. Merozoite. 7-9. Intermediate period. 10, 11. Production of macro- and microgametes. 12-14. Fertilisation. 15-18. Sporogony. 19. Sporoblast. 13-14. The zygote stages are diploid, all the other stages of the life cycle are haploid.

the zygote and a very interesting early association of the gametocytes before their sexual character is externally developed.

There are some points of interest in Naville's account of *Klossia helicina* (1927) where the cycle takes place in the liver of snails (Text-fig. 8). There are two generations of schizogony differing a little in size. The schizont nucleus is without a karyosome. A very curious feature of this cycle is the intermediate development which occurs between the second cycle of schizogony and the production of gametocytes. These merozoites, which now have a karyosome, divide usually twice

in succession, sometimes only once, and either four or two gametocytes are formed. The mitotic figures in this intermediate stage show four chromosomes (the haploid number). Naville writes "les quatre éléments jumeaux nés de deux divisions successives d'un mérozoïte constituent les jeunes gamétocytes. Ces éléments évolueront en macrogamètes ou en microgamétocytes suivant la sexualité qu'ils acquerront." But the remarkable thing here is that the gametocytes derived apparently from one haploid merozoite develop into two different sexes. Where only two are formed they "subissent presque toujours une sexualisation en sens inverse." In the more usual case where four cells are formed from one merozoite there is usually one macrogametocyte, one microgametocyte and two "éléments satellites." We have therefore here the suggestion that from one *haploid* merozoite two fully differentiated gametocytes are produced in the course of two nuclear divisions. The present writer feels that this point in the cycle is open to question as to whether the interpretation is actually correct. The figures would apparently also bear the interpretation of a pregametocytic association or syzygy of two separate individuals, which is already known in the Coccidia; an example is given above in *Karyolysus*. The rest of the cycle of *Klossia* runs a more usual course, the microgametocyte produces a number of flagellate microgametes one of which fertilises the macrogamete lying in the same cell. A fertilisation spindle arises and the zygote shows at this period eight chromosomes, *i.e.* it is as in *Aggregata* a diploid organism only for this short duration of time. In Naville's description, differing from Dobell's findings in *Aggregata*, the chromosomes retain their identity: they are to be distinguished as a male and female group at either end of the fertilisation spindle. They unite later but without disintegrating in the synaptic stage, and they conjugate in homologous pairs, undergoing a retraction in length and becoming closely associated, to segregate again at the meiotic division of the nucleus, which is the first nuclear division of the sporont.

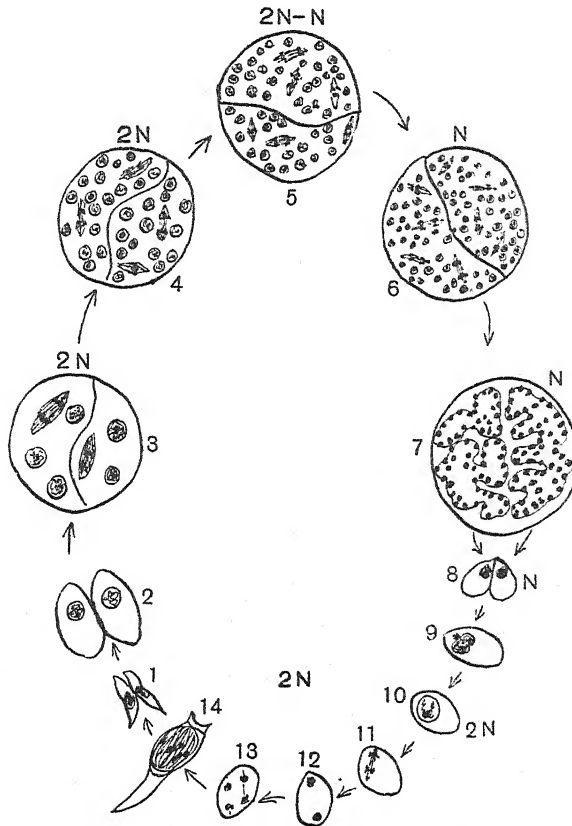
In these coccidian cycles there is a very high degree of sexual differentiation between the gametes. The motile sperm-like microgamete fertilises an egg cell macrogamete which then undergoes an active process of multiple division forming a mass of cells which are finally to become the sporozoites. The function of the egg cell in the Metazoa is also that of forming a mass of cells, namely the embryo.

The gregarines, of which only one can be cited, have particularly interesting cycles in which the production of a large number of spores is achieved in a quite different way and the distinction between the sexes, if it can be said to exist, is of the very slightest. Copulation of gametes is a perfectly regular phenomenon throughout the group of the gregarines, but there is no striking distinction of function between the gametes, and sexual characters as expressed by male and female attributes can hardly be said to exist.

Such a cycle as that of *Urospora lagidis*, very fully worked out by Naville (1927), may be taken as a type of an Eugregarine (Text-fig. 9). The gregarine is parasitic in the sea water annelid *Lagis koremi*. The young sporozoites liberated from the cyst grow into gregarines which come together in pairs and associate for some time before they enclose themselves within a common cyst, but the two gamonts



do not fuse. Within the cyst the gametes are developed in each individual by a process of repeated nuclear division and a subsequent budding off of nucleated fragments of protoplasm. Reduction occurs at the penultimate division and the diploid number of four chromosomes is reduced by a meiotic division to two. The final mitosis is of the ordinary type, the haploid number of two chromosomes splitting longitudinally and moving to either pole. The gametes set free within



Text-fig. 9. Diagram of the life cycle of *Urospora lagidis*. (After Naville, 1927, 2.) 1. Free sporozoites. 2. Young gregarines. 3. Cyst in which the two gregarines are undergoing division but without fusion. Association cyst. 4. Nuclear division within the cyst. 5. Reduction divisions. 6. Final nuclear divisions after reduction. It is a homotypic division with the reduced number of chromosomes. 7. Formation of the gametes. 8. Copulation of the gametes. 9. Zygote. 10-14. Sporogony.

the cyst copulate in pairs, the nuclei fusing and the first division of the zygote synkaryon showing the diploid number of four chromosomes. This account of Naville's gives the nuclear behaviour in great detail and there seems little doubt that the observation of the gametic meiosis is substantially correct. Mulsow, in 1911, working with *Monocystis rostrata*, a gregarine parasitic in the earthworm, also found a gametic reduction, occurring in this case at the last division in the gamont nuclei before the actual splitting off of the gametes. Calkins and Bowling (1926) confirm

this for a species of *Monocystis* in a study directed especially to this part of the cycle. Dobell and Jameson (1915) described zygotic meiosis in *Diplocystis schneideri* and were inclined to think that this condition must be general in the group. In *Diplocystis* the haploid number of chromosomes is present all through the life of the organism and only in the zygote is the diploid number present (also Jameson, 1919). This state of affairs exists as has been noted above in all the Coccidia which have been investigated in sufficient detail. In the gregarines both types of reduction appear to be represented.

In the sporozoan cycles sketched above the sequence of forms passes through so definite and striking a range that the observer is impressed rather with the rigidity of the series than with its dependence upon environmental conditions. It was certainly the early work on these forms, particularly upon the Coccidia, that impressed the alternation of asexual and sexual reproduction and the sense of a definite closed cycle upon the views of investigators at the beginning of the century. But the life cycles of the Sporozoa are more flexible than they appear to be at first sight, and they are just as much in need of an external stimulus, or, it may be said, are as closely adapted to respond to a given set of changes in the environment as are the Protozoa discussed earlier in the article. This is very striking in the digenetic species. The sexual cycle requires the stimulus of the second host, as for instance in *Aggregata* and in *Karyolysus*, to bring it about, and without this change in the environment the sporogony does not take place.

#### CONCLUSIONS AND DISCUSSION.

In considering the cycles sketched above the most striking point is their diversity. There are simple cycles with division and certain changes of form, but without syngamy, in most flagellates and in many rhizopods (it is impossible to come to any conclusion as to whether these non-conjugating cycles are primitive or secondary) and there are delicate adjustments to external conditions and to parasitism. In the cycles showing syngamy there is the greatest diversity in the method. There may be equal gametes, paedogamy or the copulation of gametes derived from a single individual; there is the development of highly specialised conjugation and the interchange of nuclei between equal individuals in ciliates, and it is to be noted that this process is effective between recently divided individuals (*Blepharisma undulans*, Calkins, 1912), and there are also a few instances in which conjugation approaches the nature of copulation suggesting a sexual difference as in *Vorticella*. One actual cycle, that of *Dallasia frontata* (Calkins and Bowling, 1928), is said to combine both a conjugatory interchange of nuclei and a copulation of gametes at different points in its history. In the large group of the gregarines we have the association of two adult gamonts, whose asexually produced derivatives show very slight if any obvious sexual differentiation, which fuse in pairs to form large numbers of zygote sporocysts. Finally in the Coccidia there is developed a sperm and egg cell type of copulation which resembles in its broad essentials the sexual process of the Metazoa.

In all this wealth of detail we have the main antithesis with which we started, namely the cycle as dependent upon its environment and the cycle as a clear and definite sequence of forms.

The dependence upon the environment has been brought out in a great deal of experimental work, so that Hartmann, Bělař, Woodruff and his school and the workers cited above whittle away the cycle until it is non-existent. The culminating point of this process is reached in Hartmann's quite recent (1928) paper on *Amoeba proteus* (*Chaos diffluens*) in which he maintains a single amoeba in a condition of active life without even the process of division being allowed to occur, by means of a daily amputation of a portion of the protoplasm. The regeneration of the protoplasm kept the cell in equilibrium for 130 days without division. Parallel experiments showed that these amoebae if left undisturbed, or if an insufficient amount of protoplasm had been eliminated, divided quite normally even after many days (e.g. 52 in one experiment) of this inhibited type of cultivation. The cycle has here been brought to the actual vanishing point and the potential immortality of the Protozoa which exercises the German workers with an almost metaphysical interest has been theoretically upheld (Hartmann, 1921, 1924 and 1928; Jollos, 1916; Bělař, 1924 etc.). The tendency to deduce that the cycle has no significance can be traced in the workers of the American schools, though Calkins (1926) adheres to the protoplasmic exhaustion theory not very different from that of Maupas. Woodruff and Spencer (1924), Woodruff (1925 and 1927) almost show surprise that conjugation should be of biological value and trace this with interest in their detailed and careful papers.

The achievement of all these workers in getting this delicate control of the conditions of culture marks a real epoch in the biological study of the Protozoa. The point that the present writer would like to emphasise is that it is the method and kind of response to the environment that constitutes any given cycle. In the general life of the species the cycle is the expression of its power of survival under the changes that the species meets. The environment is an inevitable condition of the cycle and the particular cycle is the internal expression of the individuality of the species. The individual is unable, as it were, to drive its cycle through in a perfectly uniform surrounding, and such a hypothetical medium is probably never found for any long period in nature. But when it is produced as in the experiments noted above the cycle does actually disappear, or, in other words, the cycle is not called forth.

The contemplation of the actual cycles themselves leaves us with three extremely interesting processes, namely cell division, syngamy and the development of sex. Very briefly cell division has as its centre nuclear division, and in the Protozoa after the trying out of a large number of diverse and interesting methods, all of which show a definite concern with an equal distribution of the substance known as chromatin, the mitotic process is achieved. And this is carried through into the Metazoa as a method whose broad outlines are characteristically stereotyped.

Syngamy is essentially the fusion of two nuclei generally from two different individuals. In the first place it is not necessarily connected with differences in

sex as shown in maleness and femaleness. This is most clearly brought out in the large group of the ciliates where there is no appreciable differentiation between the conjugants. The objections to this view and certain qualifications of the main theme will be stated below in discussing the phenomenon of sex. Syngamy is not necessarily connected with the initiation of division (or of the capacity for future division) as the process of fission will go on indefinitely without this stimulus. It is not a necessary rejuvenescence, as under perfectly suitable conditions there is no senescence (Bělař, 1924; Woodruff, 1925; Hartmann, 1921, 1924; Beers, 1928, etc.). Syngamy is a form of nuclear reorganisation that replaces for a longer or shorter period one-half of the chromatin substance with chromatin from another individual. This seems to eliminate or neutralise the modifications acquired during the interconjugatory periods. The occurrence of these heritable modifications derived from the pressure of the environment seems to be well established (Jollos, 1921; Duke, 1927; Reichenow and Regendanz, 1927; Ehrlich, 1909, Gonder, 1912; Werbitzki, 1910, etc.) their disappearance upon conjugation and endomixis was well shown by Jollos in *Paramecium* (1921). It seems also to effect a redistribution of genetic factors and to rearrange the possibilities of variation. We come back indeed to Hertwig's view of 1903 that "Die Befruchtung ist kein excitatorischer, sondern ein regulatorischer Vorgang." The process has also a high biological value in raising the general efficiency of the conjugants in certain forms (Woodruff, 1925, 1927). This is however debatable as the apparent increase in activity may not actually be due to the nuclear fusion, but it is at all events clear that it has a biological survival value as carried out in the normal conditions under which it is called forth.

In considering the evolution of syngamy we are led into the difficulty of the conception that everything must be implicit in the simplest form—it is the old question of the elephant being contained in the ovum—but the principle of emergence may be called upon to help in the Protozoa just as in the development of the embryo. The interpretation of primitive isogamous syngamy as shown in the flagellates, as present in the ciliates and in certain Protophyta, and as existing in the gregarines, as a biparental nuclear fusion of + and – gametes of genetically distinct value ("polarity" of Schreiber), but without male and female sex qualities, is so strange to certain workers that they prefer to assume that all chromatin as well as being divided into vegetative chromatin and idiochromatin bears a second subdivision into male idiochromatin and female idiochromatin. The argument that there is no difference in functional character between the gametes and that therefore there is no quality of maleness or femaleness involved does not seem to have come under consideration. And Hartmann, who is the most recent exponent of this old theory of Bütschli and Schaudinn, puts it (1925) that "Die morphologischen Isogameten haben wie bei den höheren Organismen eine bestimmte männliche und weibliche Tendenz, *zugleich aber hat jede Gamete stets die beiderlei Sexualpotenzen*, also die vollen männlichen und weiblichen Geschlechtstfaktoren oder Substanzen nur offenbar in verschiedenem quantitativem Verhältnis." The isogametes are according to this view differentiated into male and female while remaining bisexual. Out of this Hartmann develops the relative sexuality theory, according to which

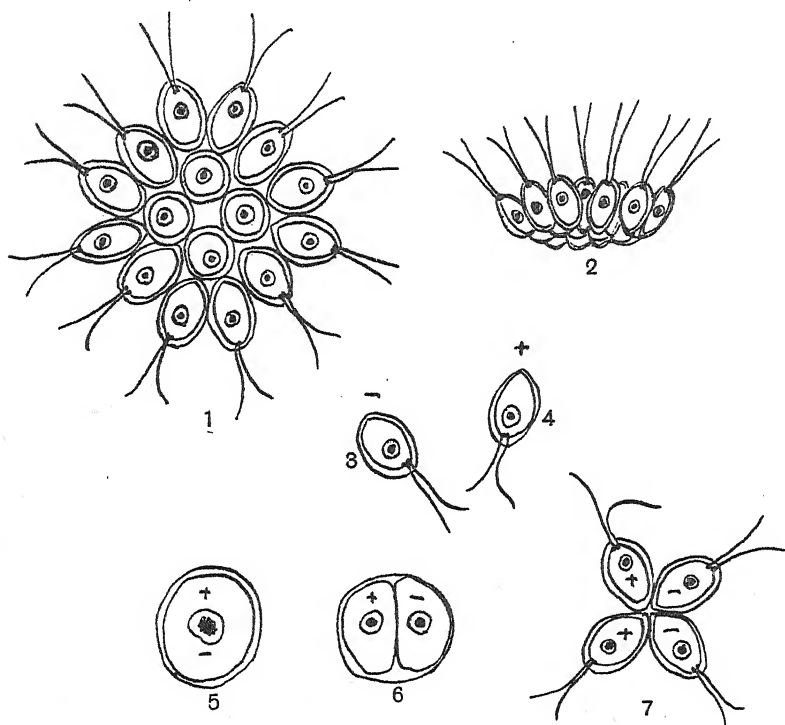
the copulation of a slightly more male with a slightly more female isogamete would occur. He considers he has an example of this in the copulation of the zoospores of *Ectocarpus siliculosus* where he finds copulation occasionally among the zoospores from one plant. The strictly dioecious nature of *Ectocarpus* is not proved beyond all doubt and Jollos (1926) points out another explanation of the facts while confirming for *Dasycladus clavaeformis* the main thesis of the occasional copulation of the gametes of one plant. Jollos finds abnormal monoecious plants in which both + and - types of swarmspores are present, one type being produced in great numbers while the other is produced in very small numbers. A low percentage of copulation between such a series of swarmspores would only be a normal +- and - copulation. The symbols + and - are used for these isogamous gametes which will only copulate with gametes of a different origin, *i.e.* of a different polarity.

A very interesting light both on this idea of relative sex and on the whole question of autogamy, paedogamy and syngamy, is thrown by a well worked out investigation by Schreiber (1925) of the life cycles of *Eudorina*, *Pandorina* and *Gonium*. The most important of these for the present purpose is *Gonium* (Text-fig. 10). He cultivates clones from single individuals (the individual is a 16-celled colony) and finds no copulation between the swarmspore gametes of one clone of this kind. Certain clones when placed together produced no copulation and no zygotes, these are of one sexual equivalent; but certain other combinations of the different clones do produce copulation; these clones are of different sex equivalents. The swarmspores of the two sex equivalents are alike and the occasional variation in the size of the isogametes is due to slight nutritional differences which may occur casually in different clones. The equal sexes are distinguished as + and -, this being the "polarity" of the gametes in Schreiber's terms. The *Gonium* is a haploid form. The zygote undergoes a heteropolar reduction in the very first nuclear division. The zygote produces a 4-celled germination colony. These cells separate, each producing a normal 16-celled adult colony and Schreiber, with great technical skill and patience, succeeded in rearing all four clones from one single germination colony. He also reared two and three clones upon different occasions. By means of clones whose polarity was already determined and by experiments at copulation of gametes between the four clones he found that two clones were + and two -. At reduction the two equal sexes are separated so that the first zygote division gives one + and one - cell. The diploid zygote is bisexual or neutral. Schreiber found that the swarmspore gametes of the one *zygote*, *i.e.* the four clones, were perfectly able to copulate + and - together. Theoretically a perfectly good copulation could here take place again after the first division between the + and - cell.

This work of Schreiber's recalls Pascher's very interesting research of 1916. He crossed two different haploid species of *Chlamydomonas* (which is a single-celled genus) and isolated the hybrid zygotes. The reduction occurs here as in *Gonium* at the first division of the nucleus of the zygote and clones derived from the single copulae were usually made up of individuals of each of the two parent species. That is the two types had separated out again at the first division of the synkaryon.



Some clones however showed recombinations of the characters giving individuals of intermediate or more correctly of mixed type, suggesting that a rearrangement of hereditary factors had taken place during the synapsis in the same manner as is now generally considered to occur in the Metazoa. Now in considering the paedogamous copulation described by Bělař in *Actinophrys* the diploid neutral vegetative form divides into two individuals and each proceeds to reduction. They



Text-fig. 10. (1 and 2 are from Stein's figures given in Doflein, 1927. 4-7 are arranged from Schreiber's account 1925.) *Gonium pectorale*, diagram of life cycle. 1 and 2. Adult colonies of 16 cells. Each colony produces gametes of one sex equivalent only. 3-4. Swarmspore gametes derived from different colonies—of differing sex equivalent. Schreiber's + and - sex polarity. The gametes are morphologically isogametes. 5. Diploid zygote containing a synkaryon made up of a + and - haploid nucleus from each gamete. 6. Reduction has taken place at first nuclear division of zygote and the sex polarity of the two cells produced is respectively + and -. 7. Germination colony containing two + cells and two - cells. Each of these cells swims away separately and grows into a 16-celled colony composed of cells of only one sex polarity.

are effective as copulae when reduced so that it would seem that one rejects the + of the diploid at reduction and the other rejects the -. When copulation has taken place the resulting zygote is once more a diploid neutral. Bělař mentions cases where the copulation fails, he suggests because of the delay in the ripening of one of the partners, but his evidence would fit equally well the case of an unsuitable maturation having produced an ineffective couple of like polarity. In the formation of the double individuals in *Heteromita* the suggestion of a paedogamous

copulation is obvious, but the nuclei are ineffective and no fusion takes place. In the haploid *Aggregata* described by Dobell sexually different gametes are produced where the male and female characters are very clear, while there are no manifest sexual differences in the schizogony where sexual function is not called into play. But reduction occurs in the first division of the bisexual zygote and in spite of the "cobweb" stage there is first conjugation and then perfect segregation of the chromosomes at meiosis. It would seem probable that the power to mature as a male or female gamete without reduction might be already present in the haploid nuclear make-up from the start. In *Karyolysus*, also a haploid type, the early association of the gametocytes accords with this view. They are already effective copulae before sexually different characters have appeared. In the haploid *Klossia* Naville gets a picture of persisting maternal and paternal chromosomes which after conjugating at the synaptic stage segregate once more at the first division. In the gregarines the isogamous gametes are either haploid and their + and - "polarity" enables them to copulate directly or they are diploid during the gametocyte stage and have to carry out the division into + and - at the reduction division of maturation. It seems curious that both types should be represented in the gregarines and theoretically the haploid character described by Jameson (1919) and Dobell and Jameson (1915), would seem more in keeping with the early association in the cyst and the behaviour of the gametes than the diploid.

In the view of the present writer syngamy is a regulation of the nucleus: differences in sex as expressed in male and female characters are secondary developments to meet the evolving separation of function. Genetically valuable gametes as in *Gonium*, and as in the ciliates, have a + and - sex "polarity" or sex equivalent of prime importance in syngamy as a regulatory process, irrespective in these isogamous cases of obvious male and female characters. Diploid states are sexually neutral or potentially bisexual (as in the Metazoa) and diploid cells do not as a rule associate. Hartmann's bisexual theory and the conception of relative sexuality seems of value in considering diploid forms but is inapplicable to effective gametes which have declared for one polarity.

Endomixis might either be a real autogamy with nuclear reduction (but the authors who describe it definitely deny the nuclear fusion) or it is an abortive preparation for conjugation which never proceeds to the act of interchange because there is no reduction and the nuclei remain neutral, never acquiring an effective gametic polarity. Its biological value is the creation of a new macronucleus.

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# THE EFFECT OF THE DURATION OF LIGHT UPON THE GROWTH AND DEVELOPMENT OF THE PLANT

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(Received January 14th, 1929.)

THE study of the effect of the length of the daily light period on the economy of the plant has received increasing attention at the hands of the investigator during the last few years, and a number of papers are now available containing the results of work along these lines. The result of varying the daily light exposure from a few seconds to 24 hours both in sunlight and artificial light has been investigated by different workers under widely varying conditions and often with rather conflicting results. It is proposed in this paper to review and discuss the recorded work in the hope of elucidating some of the factors which might have contributed to the production of the results obtained.

The whole question of the effect of lighting conditions on the growth and development of the plant opens up a wide field for experimental enquiry and the subject can be considered from many different viewpoints. Both by reason of the duration of the daily light exposure and of the effects produced, previous work falls naturally into two groups. The first of these, involving the effect of very short light periods of from a few minutes to one hour daily, will not be discussed at any length as the results produced by such treatment can be looked upon as only modifications of etiolation phenomena. Such brief light exposures are insufficient to permit of normal chlorophyll development or to promote any useful degree of photosynthetic activity, and the "growth" which results is largely the expression of the redistribution of the plant's original store of food. But the results recorded from these experiments are valuable in helping to an understanding of the response to longer light periods. The effect of such short light exposures on plant growth has recently been treated by Priestley (1925, 1926), who first reviews the recent careful work of Trumpf (1924) and then deals with the results of original work at Leeds, where it was found that exposure to sunlight or to electric light (80 candle power at a distance of approximately 50 cm.) for two minutes per day produced in *Pisum sativum* and *Vicia faba* a marked expansion of the leaf lamina and a total disappearance of the characteristic "plumular hook" of the etiolated plant. The effect of 60 minutes' light per day was a noticeable shortening of internode length, a slight development of chlorophyll and marked structural modifications compared with the etiolated plant. As Brotherton and Bartlett (1918) have shown that in *Phaseolus multiflorus* the greater length of the etiolated epicotyl is due partly to an increase in the number of cells formed and partly to their greater elongation, it is probable that both these processes contributed to the production of the

shorter internodes in this case. Exposure of root stocks of *Pelargonium* and *Polygonum cuspidatum* to two hours' sunlight per day produced plants that differed little from the normal plant in appearance except for a poor development of chlorophyll. For the nature of the light effect in the very brief exposures, Priestley suggests that a photocatalytic action is involved, as a result of which lipid substances occurring in the walls of the young vacuolating cells behind the apex are set free to diffuse outwards and accumulate in contact with the air to form a cuticle. The development of the apical shoot meristem would be thus affected, for in darkness the walls of these vacuolating cells between the meristem and the termination of the vascular strand, retain their fatty and protein substances and are thus rendered impermeable. In this way the meristem is prevented from obtaining a free supply of the nutrient substances ascending the vascular strand and less growth is possible in the superficial layers. A further effect of the retention of these impregnating substances in the walls of this tissue, with the accompanying impedance of water movement along them, is to prevent the normal vacuolation and elongation of the cells. Both these effects produce marked modifications of the normal course of stem and leaf development.

The other group of work, involving the reaction of the plant to daily light periods ranging from a few hours to 24 hours, has been largely in the hands of American workers, of whom Garner and Allard may be regarded as the pioneers. They introduced the terms "photoperiod" and "photoperiodism" to designate the length of day to which the plant is subjected and its response to that day length. Their work was related chiefly to the effect produced on the vegetative and reproductive activities of the plant by artificially lengthening or shortening the normal day. These two phases of the plant's existence are more or less antithetical and it is well known that the onset of sexual reproduction is often marked by a gradual declension of vegetative activities which in the annual and biennial plant usually culminates in death. Conversely, when flowering is held in abeyance in such plants, vegetative growth may proceed unchecked. Garner and Allard (1920) found that the majority of the species they investigated could be placed in one of two groups in regard to the response they made in reproductive activity to varying light conditions. One group they called "short day" plants in which the flowering stage was accelerated by a relatively short daily light exposure and delayed or inhibited by a long one. In contrast to these there was a much smaller group, the "long day" plants, in which a lengthened daily light exposure hastened the onset of flowering, whilst a short exposure retarded it. The inducement of precocious flowering in the "short day" plant sets a limit to vegetative activity and under these conditions relatively little growth is made, whilst when the day length is unsuitable for flower production abnormal vegetative growth takes place which might lead, if other conditions are favourable, to "gigantism." In the "long day" plants where flowering occurs earlier in lengthened days, vegetative growth is not curtailed thereby, so that in both "short" and "long day" plants the amount of growth made was roughly proportional to the light exposure.

But the subject is too confused and complex to permit of such a simple division as this, for undoubtedly other factors such as temperature, air humidity and nutrition play an important part in regulating the photoperiodic response, and there are also

many species in which growth and flowering appear to be unaffected by day length. Many of the most successful weeds, such as groundsel (*Senecio vulgaris*), chickweed (*Stellaria media*) and *Poa annua*, may be found in flower at any period of the year, and, in the vicinity of Aberdeen, gorse (*Ulex europeus*) can be found in flower at any time from November to July. Roses, carnations, pelargoniums and many other plants will flower at any season under suitable temperature and cultural conditions. Bulbs of *Lilium longiflorum* and *L. lancifolium*, and "crowns" of *Convallaria* may be retarded for months by storage at low temperatures, and by planting at intervals and growing in a greenhouse may be made to provide a succession of flowers throughout the year. The general horticultural practice of "forcing" plants into bloom will provide many other examples of species in which the length of day is obviously not the dominant factor in inducing or inhibiting flowering. The late autumn and early winter of the year 1928 were unusually mild, with the result that, as late as December, in Aberdeenshire many garden and field plants were either prolonging their flowering or throwing up inflorescences for the second time that year. *Eschscholtzia*, *Antirrhinum*, roses and carnations were all blooming freely in the gardens, whilst in the fields young flowering culms of *Lolium perenne*, *Dactylis glomerata*, *Holcus lanatus* and *Bromus mollis* were abundant.

In Garner and Allard's experiments the daily light exposure was curtailed by placing the plants for a number of hours daily in a light-proof house, whilst "long days" were produced by subjecting them to a period of artificial light after sunset. The electric light used for this purpose only maintained the low average intensity in the vicinity of the plants of from 3 to 5 foot candles.

As will be seen from Table IV on page 193, in the majority of species under investigation the flowering period was accelerated by short days and delayed or inhibited by long days. It was also found (Garner and Allard, 1923) that in the case of annuals such as the soybean, the acceleration of the flowering period by an artificially shortened day resulted in more rapid senescence in late flowering varieties than in others, though flowering occurred at practically the same time. Also in varieties and species which normally flower late in the year, the flowering period was more noticeably accelerated by artificially shortened days than in those normally flowering at the height of summer. This again was particularly marked in soybeans. Species differed widely in the range of light exposure in which they were able to flower and in the minimum exposure that was sufficient for growth (see Table IV); buckwheat flowered in any day length of from 5 to 20 hours, whilst *Mikania* only did so in an exposure of 13 to 15 hours.

Attention was paid to many other forms of expression in relation to day length, such as tuberisation, pigment formation, character of branching and size of seeds produced. In soybeans the average weight of the individual seed was greatest in the optimum light period for blossoming. Tuber formation in tuber-bearing plants was most pronounced in a light period which was just too short for maximum vegetative growth, as under these conditions the carbohydrate manufactured is greater than in shorter light periods, and is not utilised in vegetative growth as in longer light periods. Branching was usually promoted by reducing the photoperiod from the optimum

to the sub-optimum for stem elongation. Thus *Cosmos*, *Oenothera biennis* and *Sorghum* branched more freely in a 10-hour day than in the normal summer day.

Generally throughout this extensive work little recording of temperature was carried out and no attempt at temperature control, with the exception of one group of experiments with soybeans, where plants grown in a greenhouse with the natural length of a short winter day flowered earlier than those under similar controlled conditions except that they received artificial light from sunset to midnight daily. In a similar experiment with a lower average temperature neither sets of plants flowered.

In subsequent investigations (Garner and Allard, 1925) working with *Cosmos*, a typical "short day" plant, it was shown that by suitably darkening the upper, middle or lower regions of the plant for a number of hours daily, the forcing effect of the short light period on flower production was confined to the darkened part. Conversely, if any region of the main axis was subjected to an artificially lengthened day, the usual abnormal vegetative growth ensued in this region.

Garner and Allard connect the critical length of day for flowering with the habit of the plant and regard spring and autumn flowering plants as requiring a short day, the actual season depending largely on the character of the plant. Annuals require a period of vegetative growth before reproductive activity can commence, whilst in spring flowering trees, shrubs and bulbous plants, the formation of flower buds is inaugurated in the short days of the preceding autumn. They rightly point out the importance of not confusing the formation of flower primordia with their subsequent unfolding, as the processes may be differently affected by varying light periods. Soybeans grown in a 10-hour day for more than 10 days and then transferred to a normal summer day continued to flower, whilst in a similar experiment with *Cosmos*, flower buds continued to form after the change to a long day, though only a few opened. In the examples taken from horticultural practice which were quoted earlier in this paper, the same distinction must be made between the flowering of retarded *Convallaria* "crowns" in which the embryonic inflorescence is already present, and the development of *Lilium* bulbs where the stem and flowering units have still to be organised. Garner and Allard regard this photoperiodism as an important factor in the natural distribution of plants, particularly of polar species which can only survive (produce seed) if they are able to flower in the abnormally long polar summer day.

The general conclusions arrived at in the three papers quoted above are:

1. The length of the daily light period not only affects the quantity of photosynthetic material formed, but also the use to which the plant puts it.
2. Temperature is a very important environmental factor in its effect on photoperiodism.
3. The internal water supply of the plant is of fundamental importance in photoperiodism, but variations in external supply have little effect.
4. It is indicated that the degree of hydration of living cell contents (protoplasm) is subject to very delicate regulation by light exposure.

The question of the acidity relations, carbohydrate content and water content of



plant tissue under varying light periods was examined in a further paper (Garner, Bacon and Allard, 1924) where the following conclusions are drawn:

1. "*Short day*" plants.

(a) In a long daily light exposure the resulting high vegetative growth was associated with a progressive increase of acidity (H-ion concentration), particularly in the region of the growing point.

(b) In a short daily light exposure promoting rapid flowering and curtailing vegetative growth, there was a brief period of decreased acidity followed by a moderate increase up to the commencement of flowering. Sap generally was much less acid than in (a) above. Young flower buds show a progressive increase of acidity up to the unfolding of the flowers, and the upper parts of the plant are less acid than the lower.

(c) Abrupt transference from a "long" to a "short" day caused a sudden drop in acidity in the region of the growing point about 3 to 5 days afterwards, but this was temporary and was followed by an equally rapid rise to the original level. The decrease in acidity was believed to indicate definite transition from the vegetative to the flowering condition.

2. "*Long day*" plants.

(a) In short light exposures, permitting only little growth, acidity remained low.

(b) In a long day, where the elongation of the axis is accompanied by flowering, the upper part of the developing stem is less acid than the lower.

Preliminary investigations on carbohydrate content show that transference of a "short day" plant from "long day" conditions to "short day" conditions caused a rapid increase in reducing sugars in the upper portion of the stem followed by a change to polysaccharide which immediately preceded the appearance of flowers.

Adams has repeated and extended some of Garner and Allard's work and has recorded the results of fairly extensive investigations, in some of which, however, he seems to have neglected the possible effect that his method of shortening the daily light exposure would have on other environmental conditions, and the results obtained would have been more significant indications of photoperiodic effect if greater precautions had been taken in the experimental work generally. His results are included in Table IV and are not altogether in agreement with those of Garner and Allard.

In early work (Adams, 1920) it was shown that flax grown in normal day length produced heavier plants than when shaded for a number of hours daily, with no difference in the time of flowering.

In another series (Adams, 1923) he found that with 11 diverse species, shortening the normal day length by from 1 to 5 hours usually had the effect of delaying flowering and decreasing vegetative growth. Some of the species, *e.g.* maize and soybeans, had been found by Garner and Allard to respond differently to "short" days (see Table IV), whilst it will be seen that in no case did he obtain an acceleration of flowering by artificially shortening the day length. In further work (Adams, 1924) it was found that of the four species under investigation, in three cases shortening the day length had no effect either on height of plant or time of flowering, whilst in the fourth case the darkened plant flowered later than the controls.

Another paper (Adams, 1924 *a*) contains the results of experiments on the growth of various species in total darkness and in 3, 5, 6, 10, 12, 15, 18 and 20 hours' light daily. The shorter light exposures often were insufficient for normal growth, whilst the effect of the supplementary electric light in the 18- and 20-hour day, varied in different cases, growth and reproduction being differently affected.

Experiments on the "heading out" of cereals (Adams, 1924 *b*) showed that early sowings of winter wheat and rye (before the end of March) produced ears the same season, whilst later sowings did not. Also when wheat and rye were sown in October, supplementing the daylight by artificial (electric) light caused much earlier exsertion of the ears. He concluded that light and heat are interchangeable in the plant's economy up to a point, but a basal amount of each is necessary. In his most recent work (Adams, 1925) he found that in daylight supplemented by electric light of relatively low intensity, wheat and sunflower flowered earlier and hemp later than in the normal day; whilst wheat, buckwheat and soybeans grew taller in normal light, and hemp, waxbeans and sunflower less tall. He also shaded plants of certain species at different times of the day and found that as regards height, time of flowering and weight, results were as satisfactory under 2 hours' exposure to light at midday as under 3 hours' during the morning or afternoon. He concluded that experiments on the relation of plants to light must take into consideration the duration and intensity of the light and also temperature.

Deats (1925), working on similar lines to Garner and Allard, exposed tomatoes and peppers (*a*) to a "short" day of approximately 6½ hours, (*b*) to the normal winter day at Syracuse, and (*c*) to an artificially lengthened day of 18 hours, using supplementary electric light of low intensity. The amount and rate of growth made was proportional to the length of day, though the time of flowering was differently affected in each case. Tomato flowered earlier in the "long" days (cf. Adams, 1924 *a*) and pepper earlier in the normal day; neither produced flowers in the "short" day. Anatomical studies showed that the amount of xylem, phloem, cork and starch present in the stems varied with the light exposures. From her results she concluded that the relative lengths of day and night influence the plant by a change in the C/N ratio.

The only recorded work of this nature on Cryptogams is supplied by Wann (1925), who grew *Marchantia polymorpha* under "short," normal and "long" days and found that this species responds in a similar manner to "long day" flowering plants, the formation of antheridia, archegonia and sporophytes being accelerated by the use of supplementary weak electric light.

Gilbert (1925) investigated the effect of various day lengths and temperatures on the growth and reproduction of *Xanthium pennsylvanicum*, one of the typical "short day" plants of Garner and Allard. Plants were grown in "long" days (14 hours' light) and "short" days (11 hours' light), both in high temperatures (60°-90° F.) and low temperatures (40°-70° F.), and also in continuous electric light at a temperature of 78° F. and humidity 86 per cent. The "long" and "short" days were the normal day length at the time of the year when the plants were grown. His results are summarised in Table I below.

Table I.

			Vegetative period*	Average height
High temperatures	Short day	April June	13 to 15 days	11.5 cm.
	Long day		22 days 47 days	25 cm. 25 cm.
Low temperatures	Short day	January–August April–May May–August	116 days	51 cm.
	Long day		197 days 114 days 92 days	94 cm.
Continuous light			No sign of flowering after 40 days	40.5 cm.

\* The vegetative period was the growth period to the production of the first staminate flower bud.

He concluded that the relative day length action on the plant is modified by humidity, nutrient supply and temperature, and applied to their action Blackman's theory of limiting factors. As a result of chemical analyses he found that the ratios soluble C/N and total C/N ascend as the flower primordia are formed, but as these differ so widely it is impossible to connect any single C/N ratio causally with the formation of flower initials.

In a later paper (Gilbert, 1926) he gives the results of an investigation in which *Cosmos*, *Salvia*, cotton, buckwheat and four varieties of soybeans are grown, (1) in a high temperature (75°–82° F.) with low humidity (50 per cent.) and (2) in low temperature (65°–70° F.) with high humidity (85 per cent.), all during the winter months. His results are summarised below:

Table II.

	High temperature		Low temperature	
	Growth	Reproduction	Growth	Reproduction
<i>Cosmos</i>	—	Inhibited	Taller	—
<i>Salvia</i>	No difference	Budded earlier	—	—
Cotton	Much taller	—	—	Inhibited
Buckwheat	No difference	No difference	—	—
Soybean	Taller	—	—	Inhibited

Pieters (1925) found that with *Melilotus alba* and *M. indica*, plants grown in "short" days differed but little from those in the normal day, whilst plants grown in a "long" day when daylight was supplemented by electric light of medium intensity, were much taller, the difference in height being due to increase in internode length. The first species was not grown to flowering stage, but *M. indica* flowered and fruited very early in the long daily light period.

The effect of sunlight on the growth of the date palm was investigated by Mason (1925), who found that in full sunlight leaf elongation entirely ceased, but growth could be induced at any time of the day by enclosing the plant in a darkened chamber, even when this chamber (10 ft.  $\times$  5 ft.  $\times$  5 ft.) was lighted by an electric lamp of 1800 watts. Exposure to light of short wave-length using a mercury lead-glass lamp prevented growth, therefore he concluded that the short rays of the sun's light are the inhibiting agents. He states that growth in the absence of sunlight was apparently synchronous with the closing of the stomata, with consequent checking of transpiration and increased turgescence of meristematic tissue. The experimental evidence would appear to be rather scanty for the generalisation that the inhibiting effect of sunlight on growth is due to rays of short wave-length, and it is difficult to ascribe to these a causal rôle in stomatal movement.

Wanser (1922) regards the stimulus of the daily light exposure as the important factor in determining "winter" and "spring" wheats and states that by suitably regulating the photoperiod it is possible to induce "shooting" and "heading out" irrespective of season. But "response to the stimulus may be affected and altered by temperature and nutritional factors." He believes that for most monocotyledons and some dicotyledons there are at least two critical photoperiods—one for initiating culm or stalk development from the rosette stage, and another for inducing the formation of the inflorescence.

The distribution of the eel grass (*Zostera marina*) is regarded by Setchell (1922) to be independent of a particular daily period of illumination and to be largely governed by the temperature of the sea water.

McClelland (1924), working in Porto Rico on similar lines to Garner and Allard, used *Tephrosia candida*, which under normal field conditions had a definite season of heavy blossoming in mid-autumn with a day length of approximately 12 hours. Plants were subjected to artificially shortened or lengthened days, using in the latter case supplementary electric light giving the low intensity of 250 candle-power in 24 square feet; and it was found that blossoming was inhibited by a day length of less than 10 or more than 13 hours, but was quickly induced when the long summer days were shortened to 12 hours. The tallest plants were produced by the longest light exposure, and days of normal length produced plants with longer internodes than a 10-hour day, the difference in internode length corresponding to the difference in light period in the two cases throughout the year. No particulars are given of temperature or humidity.

Two investigations have been made by Tincker on the effect of a shortened daily light period on growth and reproduction. As a result of the first (Tincker, 1925) he classified the species under trial into the following five groups according to the response made in the "short" and normal day. (1) Ever blooming (no difference in growth or flowering). (2) "Short day" plants. (3) "Long day" plants. (4) Intermediate between (2) and (3) (growth only affected). (5) Species which did not flower under any conditions. He was surprised to find large quantities of carbohydrates in plants subjected to only 6 and 9 hours' daylight and suggested that the long dark period might affect the later stages of carbon assimilation as the plant "would have a longer time

to carry out condensation reactions" and so "may be able to remove the end products of the initial reactions completely," starting the "next day with a mechanism unhampered by the presence of its own products." It is also suggested that the reduced transpiration in "short" days might reduce the nitrogen available and so limit leafy growth.

In the next paper (Tincker, 1928) the results of a more extended investigation are given, and in certain cases the results of dry weight estimations and chemical analyses under the two light periods. It was found that cocksfoot and timothy, like other graminaceous plants, are retarded in growth and reproduction by short days and yielded much less stem and leaf per season. In *Helianthus tuberosus* the total dry weights per plant, above and below ground, were approximately equal under the different lighting conditions, but tuberisation was more intense in the 10-hour day (cf. Garner and Allard). Plants of *Phaseolus multiflorus* in a 10-hour day produced a greater dry weight than the control, the difference being chiefly due to the large quantities of starch stored in the thick stems and swollen roots of the "short" day plant. Tincker suggests that the length of day factor governs the utilisation of the carbohydrates manufactured and the rate of stem elongation.

In both investigations "short" days were obtained by placing the plants for the requisite number of hours daily in a ventilated, light-proof house. The temperature in the house was within 4° F. of the outside where control plants were growing, but no mention is made of humidity conditions.

An interesting and extensive investigation on the effect of varying daily light periods on plant growth and development has recently been recorded by Lubimenko and Sžeglova (1928), who grew eight selected species in 4, 6, 8, 10, 12, 14 and 16

Table III.

Daily light periods in hours which gave maximum (A) dry weight ratios, and (B) stem length, etc.

	A				B			
	Root/ whole plant	Stem/ whole plant	Leaves/ whole plant	Fruit/ whole plant	Stem length	Total leaf area per plant	Average inter- node length	Average dry weight per plant
<i>Benincasa</i>								
<i>cerifera</i>	16	*4, 16	4	8, 10	8, 16	8	—	16
<i>Momordica</i>								
<i>charantia</i>	16	4, 6	4, 16	8, 10	16	10	16	16
<i>Phaseolus</i>								
<i>vulgaris</i>	14	14	4	10	—	8	—	10
<i>Gossypium</i>								
<i>herbaceum</i>	4, 10	4, 16	6, 8	—	16	8	—	16
<i>Hordeum</i>								
<i>vulgare</i>	10	14	4	14	—	8	—	16
<i>Soja hispida</i>	4, 16	4, 16	16	8, 10	16	10	16	16
<i>Sinapis nigra</i>	8	14	4	14	—	8	—	16
<i>Papaver</i>								
<i>nudicaule</i>	10	—	—	—	—	16	—	16

\* In the cases where two light periods are given, two maxima were recorded approximately equal in value.



hours' light per day for 50-70 days in summer at latitude 60° N. The "short" days were obtained by placing the plants for the required number of hours daily in a special dark chamber whose temperature was maintained at the same level as that of the greenhouse in which they were exposed to daylight. No mention is made of the humidity of the air under the different conditions. Some of their most interesting results are summarised in Table III.

They also worked out for each species the relative amounts of dry matter formed per hour of light exposure in the different daily light periods, and this value, which may be called an "efficiency index," was greatest in each species in the following light periods:

<i>Benincasa</i>	6, 8 hours' light daily.	<i>Phaseolus</i>	8 hours' light daily.
<i>Momordica</i>	6, 8       "       "	<i>Hordeum</i>	8, 10       "       "
<i>Gossypium</i>	8       "       "	<i>Sinapis</i>	14       "       "
<i>Soja</i>	8       "       "	<i>Papaver</i>	16       "       "

Figures are also given showing the relative rates of respiration (carbon dioxide evolution) and carbon dioxide assimilation per hour, in three species after varying periods of exposure to light and to darkness, and these show considerable variations in the rate both of respiration and assimilation after such treatment.

Their conclusions include the following:

The fundamental physiological difference between "long" and "short" day plants is the specific relation existing in each case between the magnitude of the oxidation processes in respiration and of the reduction processes in the synthesis of organic substances. In "long day" plants the oxidation processes are greater in comparison with the reduction processes than in "short day" plants.

Light exposures in their effect on plant growth can be divided into three main groups.

- A. "Short" days (2-4 hours) and long dark periods.
- B. "Long" days (16 hours) and short dark periods.
- C. Equal light and dark periods.

In *A* development is curtailed by the short day length limiting the manufacture of material available for growth, and this effect is further modified by the long dark periods also limiting the photocatalytic reactions which are the basis of the chemical processes necessary for the further elaboration of the photosynthetic material; thus etiolation phenomena may manifest themselves, chiefly in modifying leaf and flower development.

In *B* carbohydrates accumulate faster than they can be utilised in further synthesis and so may cause modification of the character of the meristematic tissues at the growing points, preventing normal flower development. Such an action of long light exposures would be an indirect one on the tissues concerned.

In *C* dark and light periods are more or less equal, and leaves, flowers and fruits reach maximum development.

The reaction of the plant to different light periods varies according to the habitat of the species, for the optimum light exposure for growth and fruit production is shortest in tropical species and longest in arctic species.

Asô and Murai (1924) compared the growth of barley and peas under ordinary conditions with that made when the normal daylight was supplemented by weak electric light at night. They found that under the extra illumination the plants grew more quickly, flowered earlier and were taller and heavier.

Yoshii (1926) found that wheat, Indian millet (*Panicum milaceum*), buckwheat, sunflower, *Cosmos*, *Ipomoea purpurea*, egg plant (*Solanum melongena*) and soybean behaved as "short day" plants. Late varieties of rice (*Oryza sativa*) were also in this class but the flowering of early varieties was unaffected by day length.

Noguti (1928) also found rice (var. Aikoku) to flower earlier in a daily light period of 5 to 8 hours than in the normal summer day, whilst supplementing daylight with electric light at night inhibited flowering.

Interesting results were obtained by Schaffner (1923) on the reversal of sex in hemp grown during the short winter days, but here again there is no evidence that the lower temperature at this season did not contribute to the production of the results obtained.

Laurie (1928) gives an account of forcing asters in spring and autumn by using supplementary electric light for 3 hours at night, flowering being accelerated and increased by this means.

Knott (1926) also states (cf. Garner and Allard) that the photoperiodic response in *Cosmos* could be strictly localised, for branch tips covered with black silk bloomed some weeks earlier than uncovered ones. Catalase was noticeably less in the short day buds.

In the work so far considered the plants under investigation were grown in sunlight, "short" days being obtained by using light-proof screens or chambers, and "long" days by the supplementary use of electric light, usually of very low intensity. Garner and Allard (1920 *a*) estimated the intensity of the light employed by them as one-thousandth that of sunlight. It is evident that as a critical comparative study this work loses some of its value, for it is impossible correctly to assess the effect of a factor whose intensity is subject to such variations, which were not accurately recorded. Undoubtedly variation in day length does exert a profound influence upon growth and reproduction in some plants, but it is impossible from such work to attempt an allocation of the results amongst the various factors involved and to assign any to the "light duration" factor alone. In addition to the impossibility of placing on the same level the conditions of illumination in the different investigations, there was in any one experiment and between different experiments great variation in the other environmental factors such as temperature, air humidity and general conditions of nutrition, and except in a few cases data concerning these are not supplied. It is obviously impossible to obtain even approximately uniform conditions of illumination whether sunlight is used alone or in conjunction with artificial light, and under such conditions considerable differences of temperature and humidity must also exist between experimental and control plants. Thus an exact study of the effect of the length of the daily light period on plant growth can only be made by dispensing with sunlight and using artificial light entirely, for this can be kept practically constant and its intensity clearly stated. There are obvious disadvantages for this method, the two chief being the difference in the quality and composition of such light from that of

sunlight, and the difficulty of obtaining light of a sufficiently high intensity without a dangerously high temperature in the experimental room. The difference in energy value, etc., of any light used is constant for all the plants in any investigation, and actually practice has shown that plants normal in every way can be grown by this means.

Some of the earliest extensive investigations of this nature were carried out by Bonnier (1895), who grew upwards of 70 species of plants in electric light, comparing the growth of those in continuous light with those receiving a varying number of hours of darkness daily. He recorded striking modifications and general simplification of structure in continuous light, such as absence of palisade tissue in some laminae; cork was late or poorly developed; lignification of pericyclic and xylem fibres less or absent. It must be noted that in no subsequent work have Bonnier's results been repeated and it is probable that his experimental conditions generally produced abnormal, unhealthy plants.

Massart (1922), in an investigation similar to Bonnier's, found that in the ten species used, in no case was there any difference between plants grown in continuous and intermittent light.

Harvey (1922) records in a short paper the results of growing a number of species in continuous electric light of three different intensities. In the three different rooms the illumination per square foot was 32 candle-power, 68 candle-power and 16 candle-power respectively, the temperature varying in each case with the power of the lamps. A great variety of plants were raised from seed to maturity, and all grew well and many blossomed and set good seed. It is unfortunate that no control plants were grown in intermittent light for comparative purposes. The same experimental rooms were used in a second piece of work (Hendricks and Harvey, 1924), where attention was directed to the intensities of continuous light required for blooming, different light intensities being obtained by placing the plants at varying distances from the lamps. They found in the 45 species employed, big variations with regard to the minimum intensity and the range of intensity in which flowering took place.

Pfeiffer's (1926) work was recorded chiefly from the microchemical and anatomical viewpoint, though comparisons of the amount of growth are given in a few cases. Plants were grown under different experimental conditions as follows:

(1) Plants were exposed to 5, 7, 12, 17, 19 and 24 hours' light daily in a room lighted only by electric light, the intensity varying from 780 foot-candles at the beginning to 352 foot-candles at the end of the experiment. The temperature throughout was 25° C. and the humidity 80 per cent. of saturation. (2) In other cases greenhouses were used in which sunlight was supplemented by electric light at night, and in one case extra carbon dioxide was supplied to the air of the house. In the electric light room she found that as the daily light period was increased beyond 17 hours, tomatoes were progressively shorter and poorer in appearance with yellowing and loss of leaves, but buckwheat was better able to stand the abnormally long day, although in both cases maximum diameter of stem was attained in 17 hours per day. In a light period of less than 12 hours per day, poor plants were produced with small laminae. Root development was best where stem diameter was greatest. With *Mirabilis*, roots were best developed in 24 hours' light and stems in 17 hours' light per day. The experimental period was 16 weeks in the first two species and 6 weeks

in the latter. She concluded from her results that each species has its own optimum daily light period, increase of which produces no greater growth or tissue differentiation, but may reduce these. From microchemical tests it appeared that carbohydrates and proteins were both low in short light exposures, and that in very long light periods large quantities of carbohydrates are formed which the plant cannot utilise in tissue formation.

Maximow (1925) grew a variety of species in continuous and intermittent electric light and good growth was generally made under the continuous illumination. Species responded differently in their flowering under the two conditions, wheat and peas blooming in continuous light as in 12 hours' light per day, whilst buckwheat flowered later in continuous light and soybean not at all. Maximow was unable to find the marked anatomical differences as a result of continuous light that had been described by Bonnier.

An experiment on the growth of wheat in continuous electric light and under controlled constant environmental conditions is described by Sande Bakhuyzen (1928). The plants grew normally in every way and produced a good crop of ripe grain in 72 days from sowing.

In addition to the experimental methods used and described by Sande Bakhuyzen, an apparatus for the growth of plants under controlled environmental conditions is described by Davis and Hoagland (1928).

An earlier paper by the writer (Redington and Priestley, 1925) described the results of growing Michaelmas daisy (*Aster tradescantii*), Chrysanthemum (*Chrysanthemum sinense*), Pelargonium and *Polygonum cuspidatum* in electric light only, where plants were subjected to continuous light and to 16 hours' and 8 hours' light per day. Chrysanthemum and Michaelmas daisy made taller and bushier plants in continuous light than in intermittent light, but the other two species gave maximum development in 16 hours' light per day. In later experiments, as yet unpublished, pea (*Pisum sativum*), vetch (*Vicia sativa*), *Gypsophila elegans*, *Galium verum*, flax (*Linum usitatissimum*), hop (*Humulus japonicus*), hemp (*Cannabis sativa*), *Salvia splendens*, *Hibiscus manihot*, vegetable marrow (*Cucurbita pepo*), maize (*Zea mais*), cotton (*Gossypium herbaceum*), *Boehmeria nivea*, *Kleinia articulata*, *Maranta arundinacea*, and beech (*Fagus sylvatica*) were grown under similar conditions to the above, and in the majority of species much better growth was made in 16 hours' light per day than in continuous light, though beech was a notable exception to this. All the species which flowered under these conditions did so earlier in continuous light than in intermittent light.

Since this paper was written a report has appeared of further work by McClelland (1928)<sup>1</sup> on the effect of "long" and "short" days on certain economic plants. "Sweet potatoes, onions, pineapples, and beans" and "corn" and "potatoes" were grown under daily light exposures varying from 11 to 15 hours. Different varieties of onions and potatoes varied in their response to changes in the day length. Sweet potatoes and corn flowered early in shorter days. Pineapples and beans grew more vigorously in "long" days than in shorter days.

<sup>1</sup> McClelland, T. B. (1928). "Studies of the Photoperiodism of some Economic Plants." *Journ. Agr. Res.* 37, 603-628.

Table IV.

Species and family	Worker and reference	Comparison with normal day				Range of normal day length over experimental period in hrs.*	Details of temperature and humidity under experimental conditions	Experimental period	Normal habitat of species	Remarks on test plants
		Response in long day; with light period in hrs.		Response in short day; with light period in hrs.						
		Growth	Reproduction	Growth	Reproduction					
bean ( <i>Glycine soja</i> ).	Garner and Allard (1920)	—	—	7, 12. Short	Accelerated	15 →	Temperature in dark 2°-3° F. higher than outside	May, June →	Sub-trop.	—
tomato ( <i>Nicotiana tabacum</i> ).		—	—	5, 7. Increased	Accelerated	12-15-12		Mar. 6-Oct.	Sub-trop.	In 2 varieties out of 4
Solanaceae		—	—	7, 12. Short	Accelerated	14-15-13		May 13-Sept. 20	Temp.	—
ter <i>linifolius</i> .		—	—	7. Short	Much accelerated	15-15-11		June 16-Oct. 11	Sub-trop.	—
ench bean ( <i>Phaseolus vulgaris</i> ).		—	—	7. Short	Delayed	14-15-11		May 15-Oct. 15	Temp.	Rosette habit—swollen roots
Leguminosae		—	—	7. Diseased						
dish ( <i>Raphanus sativus</i> ).		—	—							
Cruciferae		—	—							
<i>ibrosia artemisiifolia</i> .		—	—	7. Short	Accelerated	15-15-14		June 3-Aug.	Temp.	—
Compositae		—	—	7. Short	—	15-15-11		June 4-Oct. 19	Temp.	—
Urtic ( <i>Urtica carota</i> ).		—	—	7. Short	Plants died	15 →		June 4 →	Temp.	—
stuce ( <i>Lactuca sativa</i> ).		—	—	7. Very poor	Prevented	12-15-13		Mar.-Sept.	Sub-trop.	—
Compositae		—	—	7. Very poor	—	15 →		June 7 →	Temp.	—
<i>bicus moscheutos</i> .		—	—	7. Slow	No difference	15-15-14		June 6-Aug.	Temp.	—
Malvaceae		—	—	7. Short	Inhibited	15-15-13		June 3-Sept.	Sub-trop.	—
abbage ( <i>Brassica oleracea capitata</i> ).		—	—	7. Less	Petaliferous flowers	15 →		June 9 →	Temp.	—
Cruciferae		—	—	7. Less				Oct. 20-Feb. 12	Temp.	—
olden Rod ( <i>Solidago juncea</i> ).		—	—	7. Less				Nov. 1-Feb. 12	Temp.	"Ever-blooming"
Compositae		—	—	7. Less				Nov. 1-Feb. 12	Sub-trop.	—
<i>itania scandens</i> .		—	—	7. Less				Nov. 1-Feb. 12	Temp.	—
Compositae		—	—	7. Less				Nov. 10-Feb. 12	Sub-trop.	—
iolet ( <i>Viola fimbriatula</i> ).		—	—	7. Less				July 11-Jan.	Temp.	—
Viola		—	—	7. Less				Oct. 31 →	Temp.	Cleistogamous flowers only
<i>is florentina</i> .		18. Increased	Accelerated	18. Increased			Night temperature 56.5° F.			
Iridaceae		18. Increased	Accelerated	18. Increased			Day temperature 75°-86° F.			
linch ( <i>Spiraea oleracea</i> ).		18. Increased	Delayed	18. Increased			Temperature in "long day" house 2°-3° lower than control			
Compositae		18. Increased	Accelerated	18. Increased						
adish ( <i>Raphanus sativus</i> ).		18. Increased	Inhibited	18. Increased						
Cruciferae		18. Tall	Decreased	18. Tall						
obacco ( <i>Nicotiana tabacum</i> ).		18. Tall	Vigorous	18. Tall						
Solanaceae		18. Tall		18. Tall						
<i>vestia refracta</i>		18. Tall		18. Tall						
Iridaceae		18. Tall		18. Tall						
<i>iola papilionacea</i> .		18. Tall		18. Tall						
Viola		18. Tall		18. Tall						

\* The first and last figures give the normal day length at the beginning and end of the experimental period respectively, and the middle figure, where present, is the maximum or minimum day length during that time. These data were obtained from *The Nautical Almanac*, 1927. H. M. Stationery Office, London.



Table IV (contd.).

Species and family	Worker and reference	Comparison with normal day				Range of normal day length over experimental period in hrs.	Details of temperature and humidity under experimental conditions	Experimental period	Normal habitat of species	Remarks on test plants	
		Response in long day; with light period in hrs.		Response in short day; with light period in hrs.							
		Growth	Reproduction	Growth	Reproduction						
Lima bean ( <i>Phaseolus lunatus</i> ). Leguminosae	Garner and Allard (1920)	18. Very tall	Inhibited	—	—	Night 60°-65° F. Day temperature 75°-86° F. Temperature in long day house 2°-3° lower than control	Oct. 18 →	Trop.	—		
Soybean ( <i>Glycine soja</i> ). Leguminosae						10-9-11	10-9-11	Nov. 1-Feb. 12	Sub-trop.	—	
<i>Bidens frondosa</i> . Compositae						10-9-11	10-9-11	Nov. 19-Feb. 12	Sub-trop.	—	
Buckwheat ( <i>Fagopyrum vulgare</i> ). Polygonaceae						10-9-11	10-9-11	Nov. 1-Feb. 12	Temp.	—	
Poinsettia ( <i>Euphorbia heterophylla</i> ). Euphorbiaceae						15-15-10	10, 12. Short	Accelerated	July 9-Nov.	Trop.	—
Lettuce ( <i>Lactuca spicata</i> ). Compositae	Garner and Allard (1923)	—	—	—	—	As described in Garner and Allard (1920)	Mar. 29-Aug. 24	Temp.	—		
Artichoke ( <i>Helianthus tuberosus</i> ). Compositae							13-15-14	No difference	Apr. 25-Oct. 1	Temp.	—
<i>Amaranthus hybridus</i> . Anarantaceae							14-15-12	Accelerated	July 8-Aug. 23	Sub-trop.	—
<i>Amaranthus hybridus</i> . Anarantaceae							15-15-14	Slightly accelerated	July 8-Aug. 23	Sub-trop.	—
Kulthi bean ( <i>Dolichos biflorus</i> ). Leguminosae							15-15-14	Accelerated	May 29-Sept. 1	Trop.	—
Maize ( <i>Zea mays</i> ). Gramineae							15-15-13	Accelerated	May 29-Aug. 24	Sub-trop.	—
Sorghum ( <i>Holcus holapensis</i> ). Gramineae							15-15-13	Accelerated	June 17-Dec. 17	Sub-trop.	—
Buckwheat ( <i>Fagopyrum vulgare</i> ). Polygonaceae							15-15-9	Accelerated	June 8 →	Temp.	—
<i>Comos sulphurea</i> . Compositae							15 →	Accelerated	Apr. 22-Oct. 14	Sub-trop.	—
<i>Liatris graminifolia</i> . Compositae							14-15-11	Accelerated	Mar. 27 →	Sub-trop.	—
<i>Hibiscus sabdariffa</i> . Malvaceae							13 →	Accelerated	July 12-Nov.	Trop.	—
<i>Polygonum</i> sp. Polygonaceae							15-15-10	Accelerated	June 30-Aug. 18	Temp.	—
<i>Chrysanthemum</i> . Compositae							15-15-14	Accelerated	May 12 →	Temp.	—
<i>Dahlia</i> . Compositae							14 →	Accelerated	May 12-Sept.	Temp.	—
Hedge bindweed ( <i>Convolvulus sepium</i> ). Convolvulaceae							14-15-13	Inhibited	May 13 →	Temp.	—
Hedge bindweed ( <i>Convolvulus sepium</i> ). Convolvulaceae	14 →	No difference	May 13 →	Temp.	—						
<i>Sagittaria latifolia</i> . Alismaceae	14-15-12	Accelerated	May 3-Sept. 20	Sub-trop.	—						
<i>Impatiens biflora</i> . Balsaminaceae	14 →	Accelerated	Apr. 12 →	Sub-trop.	—						

[illegible]

Table IV (contd.).

Species and family	Worker and reference	Comparison with normal day				Range of normal day length over experimental period in hrs.	Details of temperature and humidity under experimental conditions	Experimental period	Normal habitat of species	Remarks on test plants
		Response in long day; with light period in hrs.		Response in short day; with light period in hrs.						
		Growth	Repro-duction	Growth	Repro-duction					
<i>Lespedeza stipulacea</i> . Leguminosae	Tinker (1923)	—	—	6, 9, 12. Did not thrive	—	14-17-11	As described in Tinker (1925)	April-Oct.	Sub-trop.	—
<i>Lespedeza striata</i> . Leguminosae	•	—	—	6, 9. Did not thrive	—	14-17-11	—	April-Oct.	Sub-trop.	—
<i>Lespedeza striata</i> . Leguminosae	•	—	—	12. No difference	—	14-17-11	—	April-Oct.	Sub-trop.	—
<i>Clarkia</i> . Onagraceae	•	—	—	6, 9, 12. Less	Delayed	15-11	—	Aug. 2-Oct.	Sub-trop.	—
<i>Godetia</i> . Onagraceae	•	—	—	9, 12. Less	Less	15-11	—	Aug. 2-Oct.	Sub-trop.	—
<i>Parnip (Pastinaca sativa)</i> . Umbelliferae	•	—	—	10. Less	No difference	15-17-	—	May 5 →	Temp.	—
<i>Barley (Hordeum vulgare)</i> . Gramineae	•	—	—	10. Short	Delayed	14-17-	—	April 4 →	Temp.	—
<i>Oats (Avena sativa)</i> . Gramineae	•	—	—	10. Short	Delayed 1 year	14-17-	—	—	Temp.	—
<i>Groundsel (Senecio vulgaris)</i> . Compositae	•	—	—	10. Short	No difference	12-17-	—	Mar. 3 →	Temp.	—
<i>Artichoke (Helianthus tuberosus)</i> . Compositae	•	—	—	10. Short	—	15-17-13	—	May-Sept.	Temp.	—
<i>Runner bean (Phaseolus multiflorus)</i> . Leguminosae	•	—	—	5, 10. Short	Delayed	15-17-	—	May 6 →	Sub-trop.	—
Tomato ( <i>Lycopersicon esculentum</i> ). Solanaceae	Deats (1925)	18. Tall	Accelerated	6-5. Short	Sparse	11 →	—	Oct. 14 →	Trop.	—
Pepper ( <i>Capasium annuum</i> ). Solanaceae	•	18. Tall	No difference	6-5. Poor	Inhibited	12 →	—	Sept. 16 →	Trop.	—
<i>Marchantia polymorpha</i> . •	Wann (1925)	Normal plus 6. Vigorous	Accelerated	—	—	—	—	Oct. →	Temp.	—
Flax ( <i>Linum usitatissimum</i> ). Linaceae	Adams (1920)	—	—	Normal less 2-5. Less	No difference	16-15	—	June 13-Aug. 10	Temp.	—
Flax ( <i>Linum usitatissimum</i> ). Linaceae	Adams (1923)	—	—	—	Delayed	15-16-15	Approx. 6° F. higher in dark.	May 25-July 11	Temp.	—
Wheat ( <i>Triticum vulgare</i> ). Gramineae	•	—	—	Less	Delayed	15-16-15	Humidity also higher. Darkened for approx. 3 hrs. per day for about 20 days.	May 25-July 11	Temp.	Repeated twice
Soybean ( <i>Glycine soja</i> ). Leguminosae	•	—	—	Slightly less	No difference	15-16-15	—	May 25-July 11	Sub-trop.	Repeated twice

White mustard ( <i>Sinapis alba</i> ).	•	Adams (1923)	—	—	Less	Delayed	15-16-15	Approx. 6° F. higher in tank. Humidity also higher Delayed for approx. 3 hrs. per day for about 20 days	May 23-July 5	Temp.	—
Cruciferae	•		—	—	Less	Delayed	15-16-13		May 23-Sept.	Temp.	Repeated once
Sunflower ( <i>Helianthus</i> sp.).	•		—	—	Less	Delayed	—		—	Sub-trop.	Repeated twice
M. Compositae	•		—	—	Normal less 2, 3. No difference	No difference	—		-July 23	Sub-trop.	—
Citrus	•		—	—	Normal	Delayed	—		-July 25	Sub-trop.	—
Waxbean ( <i>Phaseolus vulgaris</i> ).	•		—	—	Normal less 5	Delayed	—		June 20-Mar.	Temp.	—
Leguminosae	•		—	—	Less	No difference	—		June 20-Mar.	Temp.	—
Waxbean ( <i>Phaseolus vulgaris</i> ).	•		—	—	—	—	—		—	—	—
Leguminosae	•		—	—	—	—	—		—	—	—
Liver leaf ( <i>Hepatica acutiloba</i> ).	•		—	—	—	—	—		—	—	—
Ranunculaceae	•		—	—	—	—	—		—	—	—
<i>Tiarella cordifolia</i> .	•		—	—	—	—	—		—	—	—
Saxifragaceae	•		—	—	—	—	—		—	—	—
Wheat ( <i>Triticum vulgare</i> ), Spring.	•	Adams (1924 a)	18. Tall	Accelerated	—	—	12-16	Electric light all night for 5 days per week unless noted	Mar. 27-June 7	—	—
Wheat ( <i>Triticum vulgare</i> ), Winter.	•		Tall	Accelerated	—	—	10-8-15		Oct. 31-May	Temp.	Repeated once
Gramineae	•		Tall	Accelerated	—	—	10-8-13		Oct. 31-Apr.	Temp.	Repeated once
Rye ( <i>Secale cereale</i> ), Winter.	•		Little difference	Accelerated	—	—	9-8-13		Nov. 24-Apr.	Temp.	Repeated once
Flax ( <i>Linum usitatissimum</i> ).	•		Tall	Accelerated	—	—	9-8-13		Nov. 24-Apr.	Temp.	—
Linaceae	•		Tall	Accelerated	—	—	8-13		Dec. 12-Apr.	Temp.	—
White mustard ( <i>Sinapis alba</i> ).	•		18. Little difference	Delayed	—	—	12-15		Mar. 21-May 8	Temp.	Repeated once
Cruciferae	•		18. Little difference	No difference	—	—	12-16		Mar. 21-June 8	Trop.	—
<i>Trifolium dubium</i> .	•		18. Little difference	Inhibited	—	—	14-16		Apr. 24-July 6	Sub-trop.	—
Leguminosae	•		18. Little difference	—	—	—	—		—	—	—
Buckwheat ( <i>Fagopyrum vulgare</i> ).	•		18. Little difference	—	—	—	—		—	—	—
Polygonaceae	•		18. Little difference	—	—	—	—		—	—	—
Tomato ( <i>Lycopersicon esculentum</i> ).	•		18. Little difference	—	—	—	—		—	—	—
Solanaceae	•		18. Little difference	—	—	—	—		—	—	—
Soybean ( <i>Glycine soja</i> ).	•	Adams (1924)	—	—	12. Little difference	No difference	15-→		May-→	Trop.	—
Leguminosae	•		—	—	12. Little difference	No difference	16-→		May-→	Sub-trop.	—
Hemp nettle ( <i>Galeopsis tetrahit</i> ).	•		—	—	12. Little difference	No difference	—		—	Temp.	—
Labatae	•		—	—	12. Little difference	Delayed	—		—	Temp.	—
<i>Trifolium dubium</i> .	•		—	—	12. Little difference	—	—		—	—	—
Leguminosae	•		—	—	—	—	—		—	—	—
Wheat ( <i>Triticum vulgare</i> ).	•	Adams (1925)	Less	Accelerated	—	—	9-8-12	Electric light all night	Nov. 15-Mar.	Temp.	—
Gramineae	•		No difference	Delayed	—	—	9-8-13		Nov. 15-Apr.	Sub-trop.	—
Hemp ( <i>Cannabis sativa</i> ).	•		Little difference	Little difference	—	—	—		—	Temp.	—
Moraceae	•		—	—	—	—	—		—	—	—
Sunflower ( <i>Helianthus</i> sp.).	•		—	—	—	—	—		—	—	—
Compositae	•		—	—	—	—	—		—	—	—
Rice ( <i>Oryza sativa</i> ).	•	Noguti (1928)	—	Delayed	5, 8	Accelerated	—		July-Oct. 15	Trop.	—
Gramineae	•		—	—	—	—	—		—	—	—

Table IV (contd.).

Species and family	Worker and reference	Comparison with normal day				Range of normal day length over experimental period in hrs.	Details of temperature and humidity under experimental conditions	Experimental period	Normal habitat of species	Remarks on test plants
		Response in long day; with light period in hrs.		Response in short day; with light period in hrs.						
		Growth	Repro-duction	Growth	Repro-duction					
Barley ( <i>Hordeum vulgare</i> ). Gramineae Peas ( <i>Pisum</i> sp.). Leguminosae	Asô (1924)	More vigorous	Accelerated	—	Accelerated	—	—	—	Temp.	—
		More vigorous	Accelerated	—	—	—	—	—	Temp.	—
Wheat ( <i>Triticum vulgare</i> ). Gramineae	Yoshii (1926)	—	—	Less	Accelerated	—	—	—	Temp.	—
Indian millet ( <i>Panicum milaceum</i> ). Gramineae		—	—	Less	Accelerated	—	—	—	Trop.	—
Buckwheat ( <i>Fagopyrum vulgare</i> ). Polygonaceae		—	—	Less	Accelerated	—	—	—	Temp.	—
Sunflower ( <i>Helianthus</i> sp.). Compositae		—	—	Less	Accelerated	—	—	—	Temp.	—
Cosmos ( <i>Cosmos</i> sp.). Compositae		—	—	Less	Accelerated	—	—	—	Sub-trop.	—
Morning glory ( <i>Ipomoea purpurea</i> ). Convolvulaceae		—	—	Less	Accelerated	—	—	—	Sub-trop.	—
Egg plant ( <i>Solanum melongena</i> ). Solanaceae		—	—	Less	Accelerated	—	—	—	Trop.	—
Soybean ( <i>Glycine soja</i> ). Leguminosae		—	—	Less	Accelerated	—	—	—	Sub-trop.	—
Rice ( <i>Oryza sativa</i> ), early var. Gramineae		—	—	Less	Accelerated	—	—	—	Trop.	—
Rice, late var. Gramineae		—	—	Less	Accelerated	—	—	—	Trop.	—
Aster ( <i>Callistephus chinensis</i> ). Compositae	Laurie (1928)	Normal plus 3 hrs.	Accelerated	—	—	—	—	Spring and Autumn	Temp.	—
<i>Melilotus indica</i> . Leguminosae	Pieters (1925)	16. Tall	Accelerated	No difference	—	10-11	—	Nov. 16-Feb. 17	Sub-trop.	—
<i>Tephrosia candida</i> . Leguminosae	McClelland (1924)	Above 13. Tall	Inhibited	Less than 10. Less	Inhibited	—	—	—	Trop.	—
<i>Pelargonium</i> . Geraniaceae	Redington and Priestley (1925)	Much less	Free	Less	Inhibited	—	All in electric light only. Long day column = continuous light. Normal day = 16 hrs., light per day = 8 hrs., light per day. Dark temp. approx. 3°C. below light, and humidity a little higher	7 months	Sub-trop.	—
<i>Polygonum cuspidatum</i> . Polygonaceae		Less	—	Tall, spindly	—	—	—	7 months	Temp.	—
<i>Chrysanthemum sinense</i> . Compositae		Vigorous	—	Much less	—	—	—	7 months	Temp.	—
<i>Aster tradescantii</i> . Compositae		Vigorous	—	Much less	—	—	—	7 months	Temp.	—



Pea ( <i>Pisum sativum</i> ).	Redington	Less	Little difference	Less	Delayed	—	Similar to above	Feb. 8-Apr. 20	Temp.	—
Leguminosae		Less	—	Poor	—	—	—	Feb. 8-May 11	Temp.	—
Vetch ( <i>Vicia sativa</i> ).		Less	Accelerated	Very poor	Inhibited	—	—	Feb. 8-Apr. 30	Temp.	—
Leguminosae		Less	Accelerated	Very poor	Inhibited	—	—	Feb. 8-May 11	Temp.	—
<i>Cynophylla elegans</i> .		Less	Accelerated	Less	Inhibited	—	—	Feb. 8-June 25	Temp.	—
Caryophyllaceae		Less	Accelerated	Less	Inhibited	—	—	Feb. 28-June 11	Temp.	—
<i>Gallium verum</i> .		Less	Accelerated	Less	—	—	—	Feb. 21-June 26	Sub-trop.	—
Rubiaceae		Tall. Not branched	—	Less	—	—	—	Feb. 8-June 29	Sub-trop.	—
Flax ( <i>Linum usitatissimum</i> ).		Less	—	—	—	—	—	Mar. 9-Aug. 1	Trop.	—
Linaceae		Little difference	—	—	—	—	—	9 weeks	Sub-trop.	Repeated
Hop ( <i>Humulus japonicus</i> ).		Less	Accelerated	Poor	—	—	—	Mar. 22-Aug. 3	Temp.	—
Malvaceae		Less	—	Poor	—	—	—	Jan. 27-Aug. 26	Trop.	—
Vegetable marrow ( <i>Cucurbita pepo</i> ).		Greatly increased	—	Did not thrive	—	—	—	Feb. 15-June 18	Sub-trop.	—
Cucurbitaceae		Less	Slightly accelerated	—	—	—	—	Feb. 16-Aug. 11	Sub-trop.	—
Beech ( <i>Fagus sylvatica</i> ).		Less	Little difference	—	—	—	—	Feb. 10-Aug. 9	Trop.	—
Fagaceae		Less	—	—	—	—	—	Jan. 24-July 20	Sub-trop.	—
Cotton ( <i>Gossypium herbaceum</i> ).		Little difference	—	—	—	—	—	Jan. 24-July 21	Sub-trop.	—
Malvaceae		Less	—	—	—	—	—			
Maize ( <i>Zea mays</i> ).		Less	—	—	—	—	—			
Gramineae		Less	—	—	—	—	—			
<i>Boehmeria nivea</i> .		Less	—	—	—	—	—			
Urticaceae		Less	—	—	—	—	—			
<i>Maranta arundinacea</i> .		Much less	—	—	—	—	—			
Marantaceae		Less	—	—	—	—	—			
<i>Kleinhia articulata</i> .		Less	Free	—	—	—	—			
Compositae		Less	—	—	—	—	—			
<i>Pelargonium</i> (variegated).		Less	—	—	—	—	—			
Geraniaceae		Less	—	—	—	—	—			

## DISCUSSION.

The results reviewed above, though exhibiting a certain amount of discordance, indicate that the length of the daily light period in which the plant is grown has a marked influence on its vegetative and reproductive activities. They also demonstrate the impossibility of attempting to predict the manner in which the plant will respond to changes in the daily light period, for this varies in different species and varieties. It is also clearly shown that other environmental factors, such as temperature, humidity and general conditions of nutrition, can exert as great an influence on growth as can light exposure, and this suggests that in designing and interpreting these investigations on photoperiodism, in many cases sufficient attention was not paid to the possible contributions that these other factors would make to the results obtained.

In addition to Gilbert's work already mentioned, some results obtained by Eaton (1924) are of interest here. Soybeans, cotton, *Cosmos* and maize were grown in a 13-hour day and subjected to different temperatures at night, *viz.* 90°, 65°, and 50° F. Soybeans came into flower much earlier in the high temperature and latest in the low, though the average height and total dry weight were greatest in the low temperature. Cotton flowered earlier in the higher temperature than in the medium, no growth being made at 50° F. *Cosmos* flowered a little earlier in the medium temperature than in the high, and maize flowered simultaneously in both high and medium temperatures.

## EFFECT OF THE DAILY LIGHT PERIOD ON VEGETATIVE GROWTH.

Generally, increasing the light exposure to about 18 hours daily results in a corresponding increase in growth, if "growth" is correctly interpreted as increase in living substance. Thus, increased height in short daily light periods of 8 hours or less, particularly in artificial light alone, would not be accompanied by increase in substance, but could be regarded as a modified etiolation effect. When grown in continuous light the response varies with the species and also with the stage of development of the plant, but in general, plants grown under such conditions make less growth than do similar plants receiving a dark period of a few hours daily. (Further data on this subject will be presented in a paper to be published shortly.)

A discussion on the effect of light on the development of the plant necessarily involves a preliminary consideration of the general principles of plant growth. Growth as we know it is the outward expression of two main processes in the growing organ, which are complementary to, but quite distinct from one another. Firstly is involved the fundamental growth process where an active synthetic metabolism results in the formation of new protoplasm, and hence new cells, at the growing centres or meristems. Secondly there is the supplementary process of the subsequent elongation and general increase in size of the cells so formed; this may also be looked upon as the first step in the process of differentiation which is responsible for the adult form and size of the cell. These two components of growth must be kept in mind in considering the growth of any part of the plant, for the same general routine is involved, wherever

the meristem is situated and no matter what is the tissue for whose formation it is primarily responsible. Examining in more detail, we see that the first process, cell formation, is conditioned by and is dependent upon the free supply of organic nutrients to the synthesising protoplast; whilst the second process is mainly concerned with the provision of an adequate supply of water, whose entry into the cells is accompanied by their vacuolation and elongation. These latter processes are also related to the accompanying change in the composition of the wall bounding the protoplast, which is further transformed as the result of the deposition of a cellulose layer. The conditions obtaining at the root and shoot meristems have been extensively investigated by Priestley (1928), to whose work reference may be made. Synthesis of protoplasm is not however confined to the meristem cell in its non-vacuolated state, and the entry of water and the appearance of visible vacuoles in such a cell do not involve a complete cessation of its meristematic activities, as is clearly indicated in Schüepf's (1926) recent monograph. Apparently cells of the vacuolated type left behind by the advancing meristem continue to show a limited amount of synthetic activity and divide several times. From the writer's observations it appears that this type of meristematic cell may contribute more to growth of the stem than the "intercalary" or "internodal" meristems so freely invoked and so rarely demonstrated.

In attempting an analysis and discussion of the above results, then, it is necessary to think in terms of meristems, and it is only by considering the relevant physiological and anatomical processes and their relation to conditions obtaining at the meristem that we can hope to arrive at an understanding of any growth problem. Thus the response that the plant makes to varying lighting conditions will be governed by the effects produced on these two components of growth, cell formation and cell differentiation.

#### CELL FORMATION.

The supply of food materials to any meristem of the growing plant will primarily depend on the amounts of organic material manufactured by the leaves, and of mineral matter taken in by the roots. These will then require translocation through the plant and combination to form suitable protoplasmic raw materials with their delivery to the synthesising protoplasts. Any of these processes may act as a limiting factor in cell formation, thus governing the rate at which growth can proceed. The two main physiological processes that will be affected by light are photosynthesis and transpiration, and it is only reasonable to assume that both of these will proceed to a greater extent in a long daily light period than in a short one. In the continuous light plant with adequate water supply to the roots and little variation of temperature or humidity, photosynthesis should proceed unchecked throughout the 24 hours. Thus in general starch will be formed much more abundantly than in the intermittent light plant, though this will be the case only so long as the effective leaf area of the plant is maintained at normal. An ample water supply to the continuous light plant is obviously of great importance if photosynthesis is to be maintained, for conditions of water strain in the plant may immediately be reflected in partial or complete closing of the stomata.

The cyclic reduction of the effective leaf area in plants grown in continuous light (see Redington and Priestley, *loc. cit.*) is a common phenomenon occurring in most species to a greater or less degree. In all cases noted by the writer leaves of such plants reach maturity more rapidly than those of corresponding plants in intermittent light, though these usually attain a larger size. This short growth period is usually followed by a relatively rapid withering and falling of the leaves, this having been particularly noted in *Pelargonium*, *Humulus* and *Boehmeria*. As previously noted, in some cases this feature attains a periodic regularity, a period of rapid leaf production gradually giving place to a period of slow growth to be followed by leaf fall, when often half the mature leaves will drop within a few days; the water strain being now released, active leaf production starts the cycle again. It was also noted in these cases that large leaf area coincides in time with slow stem growth and small leaf area with more rapid stem growth. This phenomenon of leaf area reduction, though affecting directly the rate of starch formation, is undoubtedly bound up causally with questions of water supply, and it is interesting to note that in two allied genera, *Boehmeria* and *Humulus*, the attainment of maturity by the leaf is accompanied by the formation of abundant tyloses in the vessels of petiole and lamina. In these extreme cases of leaf fall it is possible that this is a partial explanation of the poor growth made in continuous light, though the fact that abundant starch is usually found in the stem indicates that raw organic material is not lacking.

The question of the intake of mineral nutrients by the roots is of interest, though unfortunately few precise data are available on this point. Analyses of comparable mature maize plants<sup>1</sup> showed that those grown in continuous light had a much lower percentage of ash in the dry matter than those grown in intermittent light. Also preliminary investigations in this Department by Miss Margaret Hutchison have shown that a markedly low ash content is found in the roots of barley grown in continuous light. It is difficult to account for this ash deficiency in continuous light plants. It certainly shows that there is no quantitative relationship between the amounts of water and salts absorbed, for the continuous light plant undoubtedly takes up and transpires much more water during its life than the corresponding intermittent light plant. The fact that the processes by which water enters the plant are quite distinct from those by which salts enter has been recently pointed out by several writers (Comber, 1922), (Scott and Priestley, 1928). It seems possible that the low ash content indicates a deficiency of root action in continuous light, though whether this would be due to less root growth or to the inability of a normal root system to function properly, cannot be stated. (It is hoped that some light will be thrown on this question by further investigations which are now proceeding.) As far as visual observation goes it appears that root development, like stem development, varies in continuous light during the life of the plant.

There now arises the possibility that the actual supply of nutrients to the protoplasts of the root and/or shoot meristems is deficient, so the path of the food substances must be traced back through the supply channels to the places of manufacture, *i.e.* an

<sup>1</sup> I am indebted to Mr Godden of the Rowett Research Institute on Animal Nutrition for these analyses.

attempt must be made to find the weak link in the chain that stretches from the meristem to the leaf. Under normal conditions the starch manufactured in the leaf is changed to soluble form, probably to hexose sugars, and translocated as such through the plant to tissues requiring it, whether for burning up in respiration, retransformation into starch for storage purposes or combination with nitrogenous and other substances to form the raw material (Bausteine) of protoplasm. It might be thought that conditions of continuous illumination would interfere with this process, and such failure to remove the manufactured product would inevitably cause a slowing down of the process of manufacture. Anatomical investigations tend to dispose of this possibility, as the stems of continuous light plants which normally store starch are always packed with grains, containing more than intermittent light plants. The food material then is being manufactured and removed from the leaves, so the weakness must be in the mechanism of its supply to the meristem, and in fact the excessive accumulation of starch in the stems in continuous light tends to show that such is the case. That there is ample food available for new growth is shown particularly in *Pelargonium*, where, during an experimental period of 22 weeks, three plants in continuous light formed 7, 8 and 9 flower trusses respectively, whilst two control plants in 16 hours' light per day formed only 2 and 4. The raw materials for growth are brought up to the polar meristems in the vascular bundles which terminate some distance behind the apex, the intervening space being occupied by a band of vacuolating and differentiating tissue across which the food substances must pass. So the final channels of communication to the protoplasts of the meristem are the walls of these cells, which must be freely irrigated by the sap if the nutrients that it contains are to be readily available for these protoplasts. The two forces instrumental in conveying sap up the plant are transpiration and root pressure (Priestley, 1920; Scott and Priestley, 1928). As a result of transpiration, sap is drawn up the plant to the transpiring surfaces, and when the rate at which water is being lost from the leaves tends to exceed that at which it is being taken in by the roots, then the sap in the vascular system will be under tension or under a negative pressure, and water may even be withdrawn from other parts of the plant such as the walls of the cortex and of the differentiating tissue behind the growing points. When transpiration is at a minimum during the hours of darkness, root absorption proceeds as usual and the sap will be under positive pressure, thus ensuring the necessary supply of food materials to the meristems. It is probable that along these lines lies the chief reason for the majority of plants making more growth in intermittent than in continuous light. Continuous light means continuous transpiration, making the necessary supply of sap to the meristems difficult. This is in agreement with the observed facts that during the early part of its life the continuous light plant always grows more rapidly than the corresponding intermittent light plant, for at first the production of roots is relatively more rapid than the growth of the aerial system (Pearsall, 1923), enabling the plant to maintain root pressure at a higher level than later in life when root growth slows down. Thus in these early stages the continuous light plant will be able to utilise its more abundant carbohydrates and growth proceeds more rapidly than in the corresponding intermittent light plant. Further support for this theory is provided



by the noticeable acceleration in the growth rate following a reduction in the leaf area.

#### CELL ELONGATION.

The second possibility can now be considered, that conditions of continuous illumination prevent normal elongation of cells leaving the meristematic state. If such is the case it will be revealed by differences in the length of fundamental tissue cells formed under the different lighting conditions. Extensive measurements of the length of endodermal cells in the stems of flax, hemp, hop, *Salvia*, *Hibiscus* and cotton, show that in every case the average length varies inversely with the daily light period. Thus the greatest average length is found in plants grown in 8 hours' light per day whilst the shortest cells are formed in plants grown in continuous light. The factors concerned in the elongation of such vacuolating cells are (1) the supply of water; (2) the presence of osmotically active substances in the cell; and (3) the resistance of the cell wall to stretching. The action of light on these three factors can be very briefly considered.

(1) The supply of water to these cells will be affected both directly and indirectly by light. In constant bright light there may be a sufficiently rapid evaporation from these regions to bring about a state of "incipient drying" in the walls of the tissue (Priestley, 1926 *a*), which will limit cell elongation and probably also help to cause the failure of food supply to the meristem. The indirect action of continued illumination, as noted above, will be to cause a withdrawal of water from the walls as a result of excessive transpiration.

(2) These osmotically active substances accumulate in young cells just passing out of the meristematic state, their appearance indicating the change in the activity of the cell from the construction of protoplasm to the manufacture of carbohydrate substances, and causing the entry of water into the cell with the formation of vacuoles. Priestley (1926) suggests that carbohydrate supplies reaching these young cells from the vascular strand may in the absence of water be condensed to starch, but in the presence of water this will be hydrolysed to maltose. If this is so there will be corresponding differences in the osmotic power of the cells. It is also possible that long light exposures may exercise a direct action on the turgor pressure of these cells. It is obvious that the osmotic pressure of any parenchymatous cell depends entirely on the maintenance of the semipermeable nature of the protoplasmic membrane, and any increase in its permeability will affect the osmotic power of the cell. It is generally accepted that light has such an action (Atkins, 1916, p. 134), (Bayliss, 1924, p. 572 *b*), and Blackman and Paine (1918) have shown that the permeability of the cells of the pulvinus of *Mimosa pudica* is increased by exposure to light. If the vacuolating tissue under consideration is affected in a similar way, decrease of turgor pressure will result, with less elongation.

(3) The less the resistance offered by the cell wall to the internal hydrostatic pressure the greater will be the possibilities of extension in these cells, and apparently the walls possess maximum extensibility when they are in the "amyloid" stage (Ziegenspeck, 1920, 1925) in the differentiation of cellulose. It is possible that in a prolonged daily light exposure the "amyloid" state is of shorter duration than when

vacuolation takes place in longer periods of darkness, and thus less elongation will be possible.

The suggestions that have been advanced with regard to the effect of continuous light on the growth of the plant will also refer to any case in which the plant is subjected to an abnormally prolonged light exposure.

The decrease of vegetative growth in short light exposures is undoubtedly due to the prolonged darkness unduly curtailing the period available for photosynthesis, thus limiting the supply of food material available for growth. In cases where taller plants are produced under these conditions than under normal light exposures, it has been shown that the greater height is not due to greater bulk of living substance, but to increase in cell length, and must therefore be regarded as a modified etiolation effect.

The question of the effect of the light period on vegetative growth has been considered above without reference to the question of reproduction, for the results of fairly extensive work have shown that there is a very definite response in vegetative activity to light exposure, irrespective of whether the reproductive phase is affected or not. Where, however, the environmental conditions unduly accelerate the onset of the flowering stage the resulting diversion of food material will usually set a limit to vegetative growth.

#### EFFECT OF THE DAILY LIGHT PERIOD ON REPRODUCTION.

It is only within comparatively recent years that any progress has been made in the investigation of the important question of the initiation of the reproductive stage in the life of the plant. Some of the earliest significant work was that of Klebs (1918) on *Sempervivum*, who concluded that three stages necessary for flower production can be distinguished. The first stage is the physiological condition of "ripeness to flower"; secondly comes the formation of flower primordia and thirdly the elongation and differentiation of these resulting in the production of mature flowers. The first two stages depend ultimately on photosynthesis, as they are determined mainly by the balance between carbon assimilation and carbon outgo, chiefly in respiration, establishing the correct carbon/nitrogen ratio. The well-known forcing action of high temperatures on vegetative activity is due to the higher temperature coefficient of respiration which tends to delay the necessary accumulation of carbon produced by photosynthesis, thus keeping the C/N ratio too low for flowering.

Kraus and Kraybill's (1918) work on this question of the relation of the C/N ratio to reproduction in the plant is also classical and is well reviewed with other work by Knight (1924), to whose paper reference may be made.

A full investigation of the C/N ratio in the wheat plant has just been published by Hicks (1928, 1928 a), who found that in the three varieties under investigation, the C/N ratio rose steadily throughout the vegetative period, and when a sufficiently high C/N was obtained, flowering occurred. With winter wheat a much higher C/N ratio was required to produce flowers than in the case of a spring wheat, but in every case the C/N ratio associated with flowering represented the maximum of the ascending C/N ratio curve.

In view of this work it is inevitable that in practically all cases where attempts have been made to explain the effect of the photoperiod on flowering, the suggestion has been put forward that modifying the light exposure with the consequent control of photosynthesis affects the initiation of flowering by producing changes in the C/N ratio. Although the actual experimental evidence in support of this hypothesis is very slight it would seem a promising line of attack, although due regard must be paid to the evidence for the strict localisation of the photoperiodic response that has been put forward by Garner and Allard and by Knott. The difficulty in formulating along these lines a possible explanation of the effect of the daily light period on reproduction, is the distinction that must be made between the "long day" and "short day" plant. Where the plant flowers earlier in a "long" day it is reasonable to suppose that the abnormal light period increases the amount of carbohydrate material manufactured in photosynthesis, thus establishing the correct C/N ratio at an earlier stage than would otherwise be possible. Where, however, "short" days accelerate flowering, it is difficult to understand how the consequent reduction in the amount of photosynthetic material formed can produce the favourable C/N ratio at any earlier stage, as it appears that this ratio progressively increases with the age of the plant. If this be so the correct ratio must be slower in developing where the plant is grown from the beginning of its life in a short daily light exposure, and must already have been established in cases where well-developed plants are subjected to "short" days. Thus it appears that the stimulating action of the longer dark period on flowering must be related not to the earlier stages of "ripeness to flower" and the formation of flower primordia, but to the final stage in which the cells of the apparently dormant initials are caused to elongate and differentiate with the production of mature flowers. If this effect can be strictly localised, the action must be a direct one on the tissues concerned and photocatalytic in nature.

Also the methods of shading that were adopted in these cases must have an effect on the temperature and humidity of the air surrounding the plant, and one cannot neglect the possibility that this contributed in some measure to the production of the result obtained. A report is just to hand of an interesting paper by Bewley (1928) who stresses the necessity for the full consideration of all environmental factors in relation to their effect on the growth and development of the plant. In tomato culture light exposure must be considered in relation to temperature, and in young plants during short winter days differences of temperature of the order of 3° F. produce marked effects on the subsequent growth and development of the plant. In later stages a variation in temperature of 5° F. might affect fruit production to the extent of 28 per cent.

In the present state of our knowledge, further speculation would be unprofitable, and in conclusion it is only possible to stress again the need for further complete physiological, chemical and anatomical investigations carried out under carefully controlled experimental conditions.

Thanks are due to Professor J. H. Priestley for looking over the MSS. of this paper, and to Professor W. G. Craib for suggestions made during the course of many discussions.

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## THE GENETICS OF UNLIKE RECIPROCAL HYBRIDS

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(Received January 16th, 1929.)

A GENETICAL difference between the male and female gametes of a hermaphrodite plant has often been recorded, and it is the aim of this review to give an account of the various explanations of the phenomenon found by Mendelian analysis. Such differences in the properties of the sexual cells are often seen in the first generation from reciprocal crosses, but may not appear until the second generation is raised. In most of the cases to be referred to here the differentiating characters are not distributed as a mosaic in the plant, and therefore they do not appear to be related to those differences between reciprocal hybrids often observed, when variegation of the foliar organs is involved. In variegation the cytoplasm, or cytoplasmic inclusions, are evidently affected, and the inheritance is generally maternal; the result presumably of the direct transmission of cytoplasm through the egg<sup>1</sup>. It will however be shown that characters other than variegation may be inherited in the same way and are therefore determined by the cytoplasm or by the relation between cytoplasm and nucleus.

For the condition in which the pollen and ovules are dissimilar in their genetical properties the term "heterogametic" was used by de Vries and others, as opposed to "isogametic" in which they are alike. These expressions were however in common use in several other senses, in zoology, in botany, and also in genetics, and a change was proposed by Bateson, in the following words:

"Pending clear evidence as to the nature of the phenomenon, it may perhaps be best described as *anisogeny*, a term which merely declares that the contributions of the two sexes are unequal, in contrast to the usual *isogenous* condition, in which they are alike" (1926). In the context Bateson expresses clearly his inclination to regard the problem as one of somatic segregation, the genetical differentiation having taken place before the reduction division. In view of the recent development in our knowledge of the phenomenon, it is perhaps desirable that the use of these terms should be abandoned.

The first case of this kind recorded was that of the double stock (1908). Miss Saunders found certain commercial strains consisting of single and double-flowered plants (the latter totally sterile). In these strains all the singles gave on self-fertilisation single and double offspring in approximately equal numbers. By making reciprocal crosses between these single plants and pure breeding singles of other strains she showed that their egg-cells carry the factor for doubleness and singleness respectively, in about equal numbers, but all the pollen grains without exception

<sup>1</sup> That the inheritance of certain types of variegation might be brought about by the transmission of cytoplasm through the male gametophyte was shown by Baur (1909) and Renner (1924).

transmit the factor for doubleness. Consequently on self-fertilisation these singles give about equal numbers of double and single plants, and a permanent race consisting of heterozygous singles and sterile doubles is maintained. In the same year de Vries reported the first example of this phenomenon in *Oenothera*, to be followed by many such cases in species and hybrids of this genus, and in other plants. Later (1911) de Vries made the suggestion that the missing classes of male gametes were represented in the hybrids in question by defective pollen grains. A difficulty in this interpretation was however the fact that in some cases the female gametes were also uniform in gametic constitution, although of a different class to the male gametes. Hence the hypothesis involved the assumptions not only that deficient ovules were formed, but also that the gametic complex viable in the pollen was non-viable in the ovules, and *vice versa*. Further, in some cases deficient pollen grains were not observed, or not in sufficient numbers to account for the missing classes.

To Professor Renner we owe the solution of many of these problems in *Oenothera*. He undertook a detailed examination of the male and female gametes, and also of the early zygotic stages, in "heterogametic" species and hybrids. At an early stage of the enquiry he found an example of segregation expressed in haploid tissue, this being the first observation of the phenomenon made in any organism (1919). The hybrid (*Lamarckiana*  $\times$  *muricata*) *gracilis* produces two kinds of pollen—one, of the same size as *muricata*, with more or less spherical starch grains, and the other larger, as in *Lamarckiana*, with spindle-shaped starch grains. Owing to the difference in the starch of the two pollens it was possible to compare the growth of their pollen tubes in the styles of flowers pollinated by this hybrid. It could be seen that those containing spindle-shaped grains grew more rapidly than those with spherical grains, and in consequence all or almost all the ovules were fertilised by the former. In accordance with this observation it is found that the greater number of the male gametes transmit *Lamarckiana* characters (of the haploid group called *velans*) and only a few transmit *muricata* characters (of the haploid group called *curvans*)<sup>1</sup>.

Having demonstrated that in a hybrid a certain type of pollen grain might fail to effect fertilisation owing to the competition of a quicker growing type, Renner investigated the wild species *muricata* which had been shown to produce eggs almost all of which bear a group of characters denoted by him as *rigens*, the rest of the eggs and all the pollen bearing the *curvans* group referred to above. He found that about half the pollen grains of *muricata* were shrivelled and obviously incapable of germination; the rest were well formed but not uniform, about half being large with spindle-shaped starch grains and the rest smaller with round grains. The former germinate freely both *in vitro* and on the stigma; the latter do not germinate in either case. Thus it

<sup>1</sup> The wild species of *Oenothera*—*Lamarckiana*, *biennis*, *muricata* and *suaveolens*, are hybrid in respect of many characters which in the reduction division remain united instead of separating and recombining (the "complex-heterozygotes" of Renner). Of nine "complexes" investigated, only one, *Hookeri*, is viable in the homozygous condition, and two, *rigens* from *muricata* and *albicans* from *biennis*, are non-viable in the male gametophyte. Renner is inclined to attribute many of the exceptional genetical features of *Oenothera* to the peculiar cytological behaviour observed by Cleland (Renner, 1925, and Cleland, 1923). Recently Schwemmle has found that the species included in the section *Eu-Oenothera* are of similar genetical constitution, in that they are "complex-heterozygotes" (1928).

was clearly shown that only one kind of pollen was effective in fertilisation, and this agreed with the genetical observation that the pollen of *muricata* transmitted only the *curvans* complex. The non-germinating but well-formed pollen Renner assumed to be carrying the *rigens* complex.

On the female side of *muricata* Renner observed a process by which fertile embryos might be produced in more than half the ovules, in spite of the fact that half the potential embryo-sac mother-cells bear a non-viable complex. In *Oenothera* a row of four megaspore cells is formed by two successive divisions from the embryo-sac mother-cell. The first of these divisions is presumably the reduction division, and consequently the two upper and the two lower cells will be equivalent, having come from equational divisions. In the homozygous species *Hookeri*, and in the "isogamous" species *Lamarckiana*, the embryo-sac is always formed from the uppermost cell of the row. But in *muricata* the embryo-sac may be formed either from the uppermost or from the lowest cell of the row. In the latter case the uppermost cell was occasionally seen to continue its growth and even to become as large as the embryo-sac, the only difference being that the nucleus failed to divide. The interpretation of these observations adopted by Renner was that in *Oenothera* the development of the embryo-sac from the uppermost cell of the tetrad is favoured by the conditions. If in *muricata* that cell is genetically of the *rigens* class the embryo-sac is formed directly from it, but if it is of the *curvans* class, competition arises between it and the lowest cell of the *rigens* class. In competition the *rigens* class almost always prevails. Thus is explained the much greater frequency of eggs bearing the *rigens* complex than of eggs bearing the *curvans* complex.

The general conclusion is reached by Renner that "heterogamy" on the female side is the result of competition between the rival complexes, but on the male side the problem is far more complicated and as yet completely analysed in a few instances only.

Thus in *Oenothera* the most striking differences between reciprocal crosses do not depend on any real distinction between the male and female nuclei formed by the reduction division, neither do they depend on a difference between the cytoplasmic constitution of the parents, but primarily on competition and on differential viability among the various complexes. In the examples now to be described the constitution of the cytoplasm is of pre-eminent importance.

Bateson and Gairdner (1921), in the course of experiments on the fibre flax (*Linum usitatissimum*, the species including both oil and fibre flaxes), found a peculiar plant growing in a patch of *Linum grandiflorum*, of procumbent habit but in all other respects evidently belonging to the *usitatissimum* group<sup>1</sup>. This plant on self-fertilisation bred true. It was crossed reciprocally with a tall white-flowered fibre flax. The  $F_1$  plants from these crosses were identical and fully fertile, but in  $F_2$  from procumbent  $\times$  tall a peculiar new form appeared, in the ratio of 1 in 4, with reduced petals, the flowers

<sup>1</sup> Miss A. E. Gairdner tells me that the procumbent form has twice appeared at the John Innes Horticultural Institution, among plants of *L. grandiflorum* (a crimson-flowered erect species much grown in gardens). Messrs Sutton and Sons have also recorded the same form among plants raised from seed of *L. grandiflorum*. It does not correspond with any known variety but presumably arises from an accidental mixture of seeds with those of *grandiflorum*.

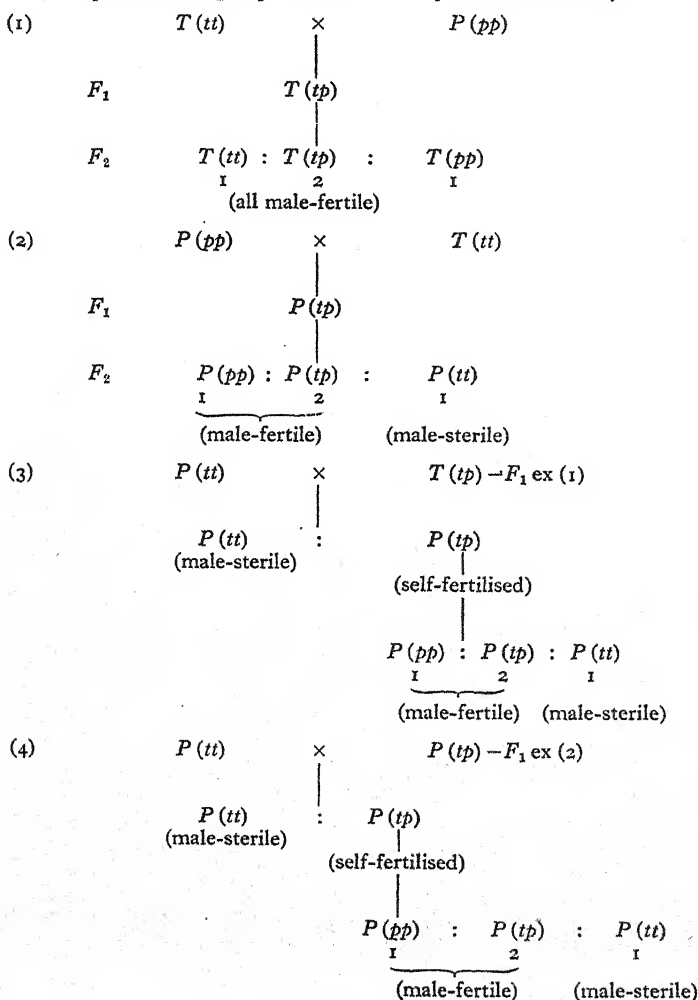
scarcely opening, and the anthers more or less aborted. These plants were called "male-steriles." The reciprocal cross gave no male steriles, either in  $F_2$  or in subsequent generations. For this reason it was at first believed that a recessive allelomorph for male-sterility had been introduced by the pollen of the tall parent, and this was confirmed by crossing back the tall variety on to the male steriles, only male-steriles being obtained. But further tests, including crosses between the male-steriles and the  $F_1$  (reciprocal) hybrids, both of which gave hermaphrodites and male-steriles in equal numbers, showed conclusively that the male-sterility occurs only when the cytoplasm of the tall variety is combined with the nucleus of the procumbent variety. The various crosses by which this relation is proved may be represented thus:

$T$  = cytoplasm of tall variety.

$P$  = cytoplasm of procumbent variety.

$t$  = factor or group of factors of the tall variety.

$p$  = factor or group of factors of the procumbent variety.



These experiments demonstrate most strikingly the stability and permanence of the original cytoplasmic distinction between the varieties. Miss A. E. Gairdner tells me that for many generations a stock of male-steriles has been maintained by crossing them with the tall variety, without any apparent change in the degree of male-sterility<sup>1</sup>. Hence that part of the cytoplasm concerned in the production of the male steriles does not appear to be carried over by the male gametophyte; further, its properties are not affected by prolonged association with the "Tail" nucleus, nor is there any reciprocal influence on the nucleus. This mutual independence is noteworthy in view of the suggestion that a parallel influence is exerted by both, in certain species hybrids in *Epilobium*.

An extensive investigation of hybrids in *Epilobium* has been carried out by Lehmann (1924) and Lehmann and Schwemmle (1927). They have found that reciprocal crosses between many of the species give identical hybrids, but when *parviflorum* or *hirsutum* are used, the reciprocal hybrids nearly always differ. Renner and Kupper (1921) had also found a difference between the reciprocal hybrids of *E. parviflorum* and *E. roseum*, which they ascribed to a cytoplasmic difference between the two species. These hybrids were closely studied by Schwemmle, who found that while all  $F_1$  plants from *parviflorum*  $\times$  *roseum* (= *rigidum*) had erect flower-spikes and were completely sterile, with greatly reduced flowers, the  $F_1$  plants from *roseum*  $\times$  *parviflorum* (= *curvata*) had drooping flower-spikes and were either *fertile*, with full-sized flowers, or *sterile*, with reduced flowers, according to the race of *roseum* used as the maternal parent. To find whether the difference among the races of *roseum* was due to a Mendelian factor (manifested only when combined with a *parviflorum* haplont) they were crossed reciprocally, and the reciprocal  $F_1$  plants were then fertilised with *parviflorum* pollen. In every case fertile and sterile plants in approximately equal numbers were obtained, showing that the male-sterility was brought about by a nuclear element subject to the reduction division. But in the families from those crosses in which the maternal parent was of the *roseum* race bearing the factor for sterility, there was an excess of sterile plants (262 fertile : 334 sterile, the reciprocal cross giving 301 fertile : 279 sterile). A possible explanation of these aberrant ratios was found on close examination of the fertile plants. Any plant capable of setting seed had been classed as fertile, but among these were all degrees of fertility, from plants with about 10 to those with about 80 seeds per capsule. On plotting the average number of seeds against the number of plants it was seen that, in the families containing a majority of completely sterile plants, the degree of fertility was lower than in the families containing a majority of fertile plants. A general shift towards sterility was in fact evident in all those families in which the cytoplasm was derived from the original *roseum* mother bearing the factor for sterility. Hence the number of steriles (50 per cent.) obtained as a result of segregation in the reduction division was increased by a certain number due to the effect of the cytoplasm in reducing fertility.

The interpretation of these experiments adopted by Lehmann and Schwemmle is that the influence of the combined haploid nuclei of *roseum* and *parviflorum*, in

<sup>1</sup> Miss Gairdner has a paper in the press recording the recent experiments on these plants (*Journ. of Gen.* 1929). Reference should also be made to Chittenden (1927).



*parviflorum* cytoplasm, causes sterility and reduction in size of the floral organs; that a similar effect may be brought about in *roseum* cytoplasm but only in the presence of a certain factor. This factor was originally derived from a race of *roseum* in which the cytoplasm was also found to have an effect on the fertility of the hybrids. For this reason Lehmann and Schwemmle are inclined to the opinion that the cytoplasm may be influenced by a nuclear element in such a way that it tends to have a similar effect on the organism. This opinion is not however based on any very extensive experimental evidence and indeed it may be questioned whether the existence of more than one type of *roseum* cytoplasm is as yet proved. In this connection the study of subsequent generations will be of the greatest interest.

In many gynodioecious plants the maternal type of inheritance which generally prevails is now recognised by Correns, who has carried out extensive experiments on these forms, as depending on cytoplasmic elements. In gynodioecious plants hermaphrodite and male-sterile individuals are found growing together (*i.e.* *Thymus serpyllum*, *Satureya hortensis*, *Campanula carpatica*, hort. vars.). In many cases intermediate degrees of male-fertility also occur and it is not uncommon to find male-sterile and fertile flowers (the latter fertile in different degrees) on the same plant. In some of these forms it has been found that the male-sterile plants give none but male-sterile offspring from seed (*e.g.* *Satureya hortensis*, Correns, 1908). In *Campanula carpatica* I have found a strain of hermaphrodites which breed true; when the male-steriles are fertilised by plants of this strain, only male-steriles are produced, and the  $F_1$  plants on crossing back to the hermaphrodite strain again give only male-steriles (Pellew, 1917). Thus the relation between the two forms corresponds exactly with that between the male-steriles ( $P(tt)$ ) and the hermaphrodite tall variety ( $T(tt)$ ), in the flax experiment of Bateson and Gairdner (p. 211). The offspring always resemble the mother, and the difference between the two forms depends on a cytoplasmic difference. Each "plasmon" (a term proposed by F. von Wettstein to denote a cytoplasm possessing distinct genetical properties) appears to retain its individual character unchanged by repeated hybridisation with other types. But between such extreme forms and the various intergrades often associated with them, a relation exists which cannot be expressed in such simple terms. The intermediate grades do not breed true; very rarely do they give regular ratios, and they are also somatically unstable. In *Campanula*, the deficient pollen of the intergrading plants is known not to be a consequence of segregation in the reduction division, for in the young anther, areas of bad grains are seen to be distributed quite irregularly (W. C. F. Newton, unpublished). Further analysis is required to discover the exact behaviour of these types, but meanwhile the suggestion that the phenomenon in general is one of cytoplasmic differentiation seems to be fully justified.

In the course of extensive and elaborate genetical studies in the mosses F. von Wettstein has found many remarkable instances of differentiation between reciprocal hybrids. He has lately discussed the significance of these differences and emphasised the importance of cytoplasmic inheritance and the great need of investigation in this field for the advancement of our understanding of allelomorphism and its relation to development (1928). The mosses are most favourable material for genetical

experiments, owing to the complex organisation of the gametophyte and also to the ease with which polyploid forms can be produced. In *Funaria hygrometrica* von Wettstein made many varietal hybrids all of which exhibited normal Mendelian behaviour; differences between reciprocal hybrids were not observed. But between the reciprocal hybrids of the two species *Funaria hygrometrica* and *F. mediterranea*, there is a well-marked difference, and moreover among the numerous segregates none were found exactly of the paternal type. In such characters as the form of the leaf-tip and the midrib, the maternal type prevailed, and this maternal influence was observed to persist into a second generation. Generic hybrids were also obtained, and that between *Physcomitrium piriforme* and *Funaria hygrometrica* was extensively investigated. The reciprocal hybrids were here identical, but again the paternal type failed to appear either in  $F_1$  or in subsequent generations. In this generic cross, the absence of the paternal type appears to be the result not only of the failure of the paternal characters to attain full development, but also to their incapacity to survive in the "foreign" (internal) environment. For whereas the specific hybrids were fully fertile, the generic hybrids were in some degree sterile, and it is possible that non-germinating spores bore the paternal (haploid) complement and that these were non-viable in combination with the maternal plasmon. In another generic cross the sterility was still greater, and only a few spores germinated, all of which gave rise to the maternal type. Here, owing to the fact that the spores of the tetrads adhere and the fate of each of the four gametophytes derived from a single pollen mother-cell can be followed, it was actually possible to demonstrate the non-viability of the paternal complements. Six fertile tetrads were observed; from each, two gametophytes died at an early stage, and two survived and developed into plants of the maternal type.

Von Wettstein has raised polyploids of the pure types and also of the various hybrids by the Marchal method of regeneration of gametophytes (chromosomes  $2n$ ) from the sporophytes ( $2n$ ). Triploids, from tetraploid  $\times$  diploid, have been subjected to the same process, and thus a very large series of polyploid forms raised, showing the effect in the hybrid sporophytes of different ratios of the parental nuclear complements. From these experiments von Wettstein makes some interesting observations on the quantitative relations of nucleus and "plasmon." Within the species *Funaria hygrometrica*, or in hybrids of species with the same "plasmon," it is clear that a gradual increase in dominance is brought about by an increase in the number of determiners of the dominant character in the nucleus. But in the experiments on the generic hybrid *Physcomitrella patens*  $\times$  *Funaria hygrometrica*, although there is a similar increase of dominance up to a certain stage, beyond that stage the degree of dominance decreases until no trace of the "dominant" character is left. In different degrees this extremely interesting and suggestive behaviour has been observed in several hybrids, in factors affecting the paraphyses, the peristome, and also the size of the cells. From it the deduction is made by von Wettstein that the inhibition of the cumulative effect in the generic hybrid is due to the influence of the "foreign" cytoplasm on the paternal nuclear complement. But although this explanation may apply to the particular case, it should be noticed that the cumulative

effect described in *Funaria hygrometrica* is hardly characteristic of polyploids in the higher plants. For instance in *Primula sinensis*, a factor exhibiting complete dominance in the diploid may in the tetraploid be incompletely dominant, although the nuclear balance is the same in both, and there is no question of a specific cytoplasmic difference (de Winton, unpublished). In *Primula* the only difference between the two plants would appear to be in the quantitative relation of cytoplasm and nucleus. Again, in *Primula sinensis*, at least one dominant factor is known having the same effect whether it is represented once, twice, three or four times in the nucleus. It seems possible therefore, that the moss hybrids are exceptional in their behaviour in these respects. Nevertheless, the observations are evidently of the greatest interest in regard to the nature and origin of factorial action and its relation to the cytoplasm.

In this brief report I have tried to indicate the state of our knowledge on the genetical significance of a difference between reciprocal hybrids. The examples described have been chosen, either because they are not explained on purely cytological grounds (*i.e.* as depending primarily on chromosome number or other chromosome aberration) or because the character affected does not appear to be directly due to the cytoplasm (as for instance does variegation of the green parts of plants). In several of the cases referred to the difference between the reciprocal hybrids has been found to rest on the non-viability of particular classes of gametes at some stage in their life-history. In no case has a nuclear distinction between the microspores and megaspores of a plant been proved to exist, but in *Oenothera*, the embryo-sac may be formed preferentially from a particular type of megaspore, to the exclusion of the rival type. A case not yet explained is that of the double stock, but the work of Snow (1925) suggests that in these plants elimination of male gametes of a particular type occurs, and the work of Frost and Mann (1924) shows that cytological irregularities are not uncommon, and may account for the phenomenon. Thus the suggestion that the phenomenon might be brought about by a segregation of allelomorphs in the soma, occurring before the formation of the male and female organs (Pellew, 1917) is no longer tenable.

In the rest of the examples quoted, the solution of the problem rests on the proof that the cytoplasm of closely related individuals may possess different properties of a permanent and stable nature, a fact established by the work of Bateson and Gairdner on flax. In the reaction between cytoplasmic systems of different types (the "plasmon" of von Wettstein) and nuclear complements of different types, lies the explanation of an alternative Mendelian or "maternal" inheritance of a single pair of allelomorphic characters.

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# DETERMINATION OF TYPES OF INDIVIDUALS IN APHIDS, ROTIFERS AND CLADOCERA<sup>1</sup>

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(Received March 8th, 1929.)

IN responding to the request of the editor for a review of the investigations into cycles involving alternating modes of reproduction, it has seemed wise to limit the field to the rotifers, aphids and cladocera. These three groups possess a cycle fundamentally alike. A unified discussion of them is therefore possible, even though a unified explanation has not yet been attained. Moreover, to treat adequately a larger field would require an article beyond any desirable limit of size.

## *The cycle.*

In all three groups, reproduction is chiefly by parthenogenesis, but at times gametic females and males are produced. By these gametic forms fertilised eggs are produced which are capable of living over winter, and, in the case of the aquatic forms (rotifers and cladocera), over periods of drought. Fertilised eggs may, of course, be produced at other times. From the fertilised egg there hatches a female which is obligatorily parthenogenetic, and her offspring are all females. The nature of these females of the second generation varies somewhat, but in each of the three groups of animals there are species in which the individuals of the second generation may be either obligatorily parthenogenetic or gametic. There are some species having a "fixed" or "closed" cycle in which the generations are not subject to variations, but naturally these species will not figure largely in the investigations about to be reviewed. In the species with "open" cycles, the successive generations consist of varying proportions of obligatorily parthenogenetic and gametic forms. The gametic forms may produce fertilised eggs and the cycle be repeated. The parthenogenetic individuals of each generation continue to produce either parthenogenetic or gametic forms as long as conditions are suitable. The combination of parthenogenesis with gametic reproduction, as it occurs in these groups, was given by Leuckart in 1865 the name of Heterogony, a term still frequently used in the European literature.

Beyond these points of likeness, there are important differences. In the rotifers, the gametic female, if not fertilised, produces males from the same eggs as would, if fertilised, produce females. The gametic females therefore precede the males by one generation, and sex is absolutely bound up with the mode of reproduction. Males are produced in no other way than by unimpregnated gametic females. The gametic and parthenogenetic female rotifers are structurally alike, and cannot be distinguished until they produce offspring, except by microscopic examination of their oocytes. In the aphids, parthenogenetic females and gametic females are usually

<sup>1</sup> Contribution from the Zoological Laboratory of the University of Michigan.



structurally distinct, though in rare cases one female may produce eggs of both kinds. Male aphids are not the sons of gamic females, but each sex is produced by certain parthenogenetic females. The parthenogenetic females of any generation, in most species, may be either winged or wingless. In most (but not all) species the gamic female is wingless and the male winged. The aphid cycle is less sharply defined, therefore, than that of the rotifers, and sex is only loosely connected with the mode of reproduction. In the cladocera, in typical cases, any female, after the one which hatches from a fertilised egg, may be either parthenogenetic or gamic or both, and if she is parthenogenetic her offspring may be either female or male.

Partly because of the presence or absence of wings, the cycle of the aphids is subject to enormous variation. The above statement with respect to them is a bare outline. Excellent statements of many of the peculiarities of their cycle are to be found in articles by Klodnitski (1912), Mordwilko (1907 *a*, 1907 *b*, 1908, 1909) and Davidson (1927 *a*, 1927 *b*).

Certain features of the cycles have been discussed by Shull (1925) with special reference to the work then most recent.

#### *Early ideas of these cycles.*

At least as early as Bonnet, in 1743, it was known that aphids could be produced without sexual union of their parents. For a long time it was supposed that this reproduction might be strictly asexual, though before the middle of the nineteenth century parthenogenesis was more than suspected. However, in 1856 it was still stoutly maintained by Burnett and by von Siebold that the young of agamic aphids were produced from internal buds. Even as late as 1866 Balbiani deemed it necessary to debate this old question, and to furnish proof of parthenogenesis by demonstrating the presence of a reproductive system comparable to that of the gamic female and by showing that the embryo develops within this system (the agamic aphids being usually viviparous) after the same general plan as in the fertilised egg. That the cell from which these offspring developed was a true egg because it had undergone maturation was not, of course, known at that time.

There was much less ground for doubt regarding parthenogenesis in the rotifers and cladocera, because in these small transparent animals the eggs are conspicuous objects. Nevertheless, even in 1906, Punnett suggested that the rotifers might really be hermaphroditic, a possibility that has since been disproven by cytological studies.

#### *External modifying factors.*

The challenge to investigation of these cycles was their ever-recurring and sometimes apparently lawless change. Gametic forms were found to be abundant at certain times, wholly wanting at others. Winged aphids appeared in droves at certain periods, but only occasionally or not at all at other periods. Naturalists have habitually attributed striking or sudden changes of all sorts to environment. Temperature and nutrition were the obviously changing features of the environment, and they have been at one time or another held responsible for most of the alterations which these cycles undergo.

*Temperature and rotifers.* The classic work of Maupas (1891) was the first to indicate experimentally an effect of temperature on the cycle of the rotifers. Rearing *Hydatina senta* at high temperatures, he obtained 97 per cent. of gamic forms, at low temperatures only 24 per cent. If the rotifers were alternated between these temperatures, more gamic females were produced at the high temperatures than at the low ones. Shull (1911) likewise obtained a temperature effect on this same species, but the relations were reversed; more gamic forms were produced at 10° C. than at 20° C. or 24° C. Shull regarded this effect as an indirect one, altering the response of the animals to some other agent, and, as is pointed out below, Nussbaum (1897) considered Maupas's results to be due to starvation, since high temperature would cause the rotifers to multiply in their cultures, devour all their food, and thereafter starve. Hartmann (1919) included high temperature among the factors inducing periods of depression, and since depression is sometimes accompanied or followed by gamic reproduction, temperature might be said to be a factor in such reproduction. Other authors have been unable to find any effect of temperature, among them Nussbaum (1897), whose criticism of Maupas has been mentioned; Punnett (1906); Whitney (1907); Noyes (1922), working with a species (*Proales decipiens*) in which she never saw gamic individuals; and Watka (1928), who studied five different species. Certain workers have found that a *change* of temperature affects gamic reproduction. Thus Moro (1915) concluded that either an increase or a decrease of temperature induces gamic forms; and according to Tauson (1926 *a*, 1927 *b*), changes of temperature, while themselves ineffective, accentuate the response of the rotifers to changes of pH (of which more is related below).

*Temperature and cladocera.* In the cladocera, Issakowitsch (1905, 1907), Papanicolau (1910 *a*, *b*, 1911), Grosvenor and Smith (1913), G. Smith (1915), and Banta and Brown (1924 *a*, 1928) have concluded that low temperature induces gamic forms, while McClendon (1910) found contrariwise that high temperature has this effect. Some of these authors regarded the temperature as an indirect agent. Thus, according to Issakowitsch, and perhaps Grosvenor and Smith, the effect of low temperature is to reduce nutrition. G. Smith showed that fat is stored at low temperatures, glycogen at high temperatures, and that the gamic forms store chiefly fat; this he believed to be the manner in which low temperature favours gamic reproduction. A retardation of metabolism, accomplished by low temperature as well as certain other factors, was believed by Banta and Brown to be the primary agent. McClendon, who attributed gamic forms to high temperature rather than low, suggested that the permeability of the plasma membrane of the eggs is reduced so that they will not develop without the added stimulus of fertilisation, a suggestion that would have more point if the gamic egg and the parthenogenetic egg were identical up to the moment of possible fertilisation, which they are not, since, as is pointed out below, their maturation processes are different. As against all the preceding workers, there are two who were unable to observe any effect of temperature, namely, Kuttner (1909) and von Scharfenberg (1914), while Green (1919) was unable to interpret the results of his temperature experiments.

*Influence of temperature on aphids.* In aphids, the effects of temperature, as of several other factors, are dual, since there are two phenomena to be influenced—the production of wings, and the stimulation of gamic reproduction. The gamic forms appear to have received the earliest attention. Kyber (1815) reared species of *Siphonophora* and *Rhopalosiphum* indoors for four years without any gamic forms, although these appeared out-of-doors in the same species at the appropriate season. Keller (1887) referred to Landois in 1867 as attributing the gamic generations to autumn conditions, but without specifying temperature in particular, and without indicating whether there was experimental evidence of such influence. Suggestions that temperature is one of the factors involved in gamic reproduction have been made by Hunter (1911), Baker and Turner (1916), Davidson (1921 c, 1927 a, b) and others, largely from the fact that low temperature prevails at the season when such reproduction occurs, and without any experimental evidence mentioned in the sources named, though Davidson (1924) definitely connected low temperatures with gamic forms of *Aphis rumicis* in experiments in which apparently no controls were maintained. Baker and Turner stated that more and more severe conditions (of which temperature is one) are required to bring on gamic forms in successive generations, but the evidence appears to be observations in nature. Klodnitski (1912) definitely held that temperature has no effect on mode of reproduction, and stated that up to that time no other factor had been shown to have such effect. However, unpublished work of Shull on *Macrosiphum solanifolii* (= *gei*) shows an unmistakable effect upon the production of gamic females. Winged mothers removed from low to high temperature produce gamic daughters at first, but gradually, over a period of ten days or two weeks, change completely to the production of parthenogenetic daughters, while similar females continued at low temperature yield almost exclusively gamic offspring. Many intermediates appear in the period of transition. The statement by Hottes (1928) that tropical species dispense with gamic forms may well find its foundation in this temperature effect.

The wings of aphids have been many times attributed at least partly to temperature. If suggestions based only on the observation of wings at times when certain temperatures prevailed be omitted, one of the earliest evidences of wing control by temperature was the work of Ewing (1916) on *Aphis avenae*, which showed the minimum of winged females produced at 65° F., with larger numbers at both higher and lower temperatures, a result confirmed by Wadley (1923). Gregory (1917), however, believed that the temperature in Ewing's experiment acted indirectly by modifying the metabolism of the host plants. Wadley, also, regards the temperature as at least partly an indirect agent, since he found that 65° F. interfered less with the wing-producing influence of starvation than other temperatures did. A similar suggestion of indirect action on nutrition in the plant appears in the paper of Mordwilko (1908). Call (1918) obtained many wings at 60–70°, none at 84–90° F. And Ackerman (1926), after extensive experiments with *Rhopalosiphum prunifoliae*, concludes that maximum wing-production is attained at two temperatures (16° and 24–26° C.), and minimum wing-production at two temperatures (12° and 18–20° C.). Ackerman also found that a *change* of temperature

was followed, after 3-10 days, by maximum wing-production, after which, under constant temperature, wings gradually decreased in number. Finally, Shull (1929) shows temperature to act indirectly upon the effects of light. Alternating light and darkness, which produce wings under ordinary conditions, do so fully only at temperatures up to 20°, in diminishing degree from 20 to 24° C., and not at all at 26° C. and higher temperatures. The nature of this interference of high temperatures with the action of light and darkness is unknown.

Not all investigators, however, have found temperature a factor in wing-production. An old example is that of A. C. F. Morgan (1885), who argued that temperatures can have no such effect on *Phylloxera*, since wings are unknown in this form in Portugal—a suggestion made in evident expectation that high temperature, rather than low, might be expected to induce wings. Others finding no effect of temperature are Shinji (1918), who experimented with chemical substances and stated that changes of temperature did not induce wings in his experiments with non-wing-developing substances; R. H. Smith (1923), who pointed out that winged individuals of the clover aphid appeared in August and September at temperatures as high as those of July; Marcovitch (1924), who believed that another factor (light) supplants the long-favoured temperature as a wing-producing agent; and Reinhard (1927), who obtained no wings in *Aphis gossypii* at any temperature from 70° to 90°.

#### Nutrition.

Probably more attempts have been made to connect the life cycle of these animals with nutrition than with any other factor, temperature, however, being a close second.

*Rotifers.* The first experimental evidence of a nutritional effect on the cycle of rotifers appears to be that of Nussbaum (1897) who, on starving *Hydatina senta*, obtained an excess of gamic females. Since he got no such effect from low temperature, he suggested that Maupas's results from temperature were really dependent on nutrition, as explained above. Further experiments with starvation by Punnett (1906) and Whitney (1907) resulted in no change of the cycle. Shull (1910 a, 1910 b) increased the number of gamic females from 25 per cent. to 36 per cent. by starvation, but regarded this as due to the smaller amount of culture water (chemical substances) introduced with the smaller amount of food. More recently Tauson (1927 b) concluded that low nutrition is the second most potent factor (pH being first), and that combined with pH it may even increase gamic reproduction to 100 per cent. Opposed to the idea of starvation as a producer of the gamic females is the work of Mitchell (1913) on *Asplanchna*, in which it was found that low nutrition excluded the gamic forms, while high nutrition produced about 20 per cent. of gamic forms. He reported that starving very young females born of poorly nourished mothers made them all parthenogenetic; but that starving those derived from well-nourished parents made many of them gamic. Shull (1913 b) doubted the validity of these results because only ten individuals were used in each test, whereas rotifers exhibit sudden and extreme changes, under ordinary conditions, and because they implied an ability to change the females during their own

lifetime, a point discussed further below. However, that high nutrition rather than low is the cause of gametic reproduction was supported by Whitney (1916), who stated that rich as against scanty food produced gametic forms in *Brachionus* and *Pedalion*; again by Whitney (1917 a), using different kinds of food and several species of rotifers; again on *Hydatina senta* by Whitney (1919), when he pointed out that under certain conditions the food organisms become inactive and are less easily obtained by the rotifers; and by Wesenberg-Lund (1923), who called attention to what has probably been observed by all investigators of rotifers, namely, that abundant gametic reproduction is preceded by rapid increase in numbers of individuals.

No effect of starvation was obtained, however, by Zawadowsky (1916), according to Luntz, or by Noyes (1922) working on a species (*Proales decipiens*) in which the gametic forms were not known, or by Watka (1928) studying several species.

The study of nutrition was given a new direction by Whitney (1914 a), when he reported that the use of the green organism *Chlamydomonas*, instead of the usual colourless *Polytoma*, produced a great excess of gametic daughters. The test was repeated later on another strain (Whitney, 1915) with the same result, and still later (Whitney, 1916) with three genera of rotifers and, in part, another genus of green organism for food. Wesenberg-Lund (1923) reported finding under an algal carpet gametic forms of species not exhibiting these forms elsewhere, a fact easily interpreted as supporting Whitney's discoveries regarding green food. In all the experimental cases the green food yielded many more gametic females than did the colourless food. The effect was at first attributed to the quality of the green food as such, but it soon appeared (Whitney, 1914 b) that mere change from *Polytoma* to *Chlamydomonas* was at least a large part of the cause. The idea that change of food, rather than a particular kind of food, favours gametic reproduction has been supported by Moro (1915); by Hodgkinson (1918), who reversed the change (from green to colourless food); by Luntz (1926), who worked with *Pterodina elliptica* and changed the food from *Chlamydomonas* to *Polytoma* or from *Polytoma* to *Chlamydomonas* with equal effect; and by Watka (1928), who obtained an increase of gametic reproduction in 60 per cent. of the experiments in which food was changed. Luntz stated that constant food, no matter which kind, yielded only parthenogenetic females, and that, if a change of food is to produce gametic forms it must be preceded by at least two successive generations with a single kind of food. He also found that change of food is effective only in a certain range of pH values, a subject discussed later.

*Nutrition in cladocera.* From experiments with nutrition de Kerhervé (1892, 1895) reported that abundant food caused continued parthenogenesis, while low nutrition induced males and gametic females. His experiments were apparently performed mostly with mass cultures. Issakowitsch (1905), with a few animals to each culture, found likewise that starvation or scant food tended to produce the gametic forms in the cladocera. Similar results with low temperature led him to conclude that the temperature reduced the nutritive processes or the supply of food, and that this was the immediate cause of gametic reproduction. He pictured the process



as follows. If a female's nutrition is slightly lowered, she produces male offspring. If her nutrition is lowered still more, the oöcytes are unable to procure the requisite material for any kind of egg, with the result that they combine in groups to produce ephippial (gamic) eggs. If an empty ephippium was produced, the next brood was always parthenogenetic, which he interpreted to mean that the egg which should have been in the ephippium had been resorbed and served as food for the next oöcytes, with the result that they were parthenogenetic. Keilhack (1906) objected to the idea of nutritive control of the cycle, on the ground that *Polyphemus pediculus*, which is dicyclic, passes through one of its gamic phases in June at a time when food is abundant. Strohl (1907) also, and for similar reasons, rejected the idea that gamic eggs result from low nutrition. Issakowitsch was supported, however, by Woltereck (1909), who based his conclusion on observations in nature; by McClendon (1910), who obtained confirmatory results from experiments; and by von Scharfenberg (1911), who reported, with respect to *Daphnia magna*, that much food results in parthenogenesis, less food in gamic reproduction, and no food in no change in the cycle. De la Vaulx (1919) obtained numerous intermediates, forms resembling both sexes in different features, at times of low nutrition, which might be interpreted to mean that a complete transition to the production of males could also be attained by low nutrition. Lack of food was listed by Hartmann (1919) as one of the factors inducing depressions, and these depressions are sometimes accompanied by gamic reproduction.

Certain authors besides Issakowitsch regarded the apparent effect of other agents as being produced through an effect on nutrition. Thus, Grosvenor and Smith (1913) held that low temperature and crowding may affect nutrition, though G. Smith (1915) was of the opinion that crowding affects accumulation of excretions, not a reduction of nutrition or the food supply. Apparently only one worker (von Scharfenberg, 1914) has reported a difference in the effect of different kinds of food. He was able to produce parthenogenetic or fertilised eggs at will in *Daphnia magna* by feeding with green algae, or with algae that had been eaten and passed by other cladocera. Finally, Kuttner (1909) and Green (1919) obtained no effect on the cycle from differences in nutrition.

*Nutrition in aphids.* Relatively little evidence is available concerning nutrition as a factor in the gamic reproduction of aphids. Until these insects can be induced to feed on artificial media, it will continue to be impossible to alter their food without introducing other factors than simple nutrition. It is possible to withhold food altogether by removing the aphids from their plants, but no one appears to have influenced gamic reproduction by this procedure. Conclusions regarding the appearance of gamic forms as a response to nutrition must be therefore largely inferences even when the evidence is obtained from experiments. Nearly every writer who discusses this phase of the cycle mentions the probability or possibility that low nutrition does induce gamic reproduction. It is possible to interpret the 4-year parthenogenesis obtained by Kyber (1815), mentioned above, as due to high nutrition. Balbiani (1866) suggested nutrition as the controlling factor. Keller (1887) stated that Düsing in 1884 held nutrition responsible for the reproductive

changes, and that Landois in 1867 attributed gamic forms to autumn conditions, of which reduced or altered food was no doubt conceived to be one. Davidson (1921 *b*, 1921 *c*) assents to the view that food conditions control gamic reproduction, and Hottès (1928) quotes Takahashi as finding only parthenogenetic aphids on young *Celtis* trees in Formosa, but some gamic forms on old trees.

On the whole, it can hardly be regarded as proven that nutrition has any such control, but this remark should not be interpreted to mean that it probably has not.

Much more information is available about the effect of nutrition upon wing-production in aphids, although this evidence also suffers from confusion of what appears to be nutrition with things which may not be nutrition. Morgan (1885) found that *Phylloxera* on grape roots that were allowed to dry and die produced many winged individuals. Keller (1887) referred to the statement by Göldi in 1885 that starvation caused wing development in *Pemphigus*. Grassi (1907) is reported to have found that wings in *Phylloxera* depended partly on whether an American or a European variety of the host plant was used. Nutrition is one of the factors given by Mordwilko (1909) as inducing wings in his experiments, but he obtained wings sometimes so quickly that one is almost driven to conclude that the real factor started earlier in those cases. Woodworth (1908) observed that wilting of the plants was followed by wing-production. Crowding of the aphids was found to be followed by wing-production by Grassi (1907) in *Phylloxera*, Davidson (1914) in *Aphis rumicis*, Wadley (1923), Ackerman (1926) in *Rhopalosiphum prunifoliae*, and Reinhard (1927) in *Aphis gossypii*, though not by Slingerland (1893), while transfer of the aphids to young plants prevented wings, according to Klodnitski (1912). Experiments with starvation by Gregory (1917), Wadley (1923), Ackerman (1926), Reinhard (1927), and Shull (1928) showed this factor to induce wings, though in the work of Shull certain light conditions influenced the results, and in that of Ackerman and of Reinhard the number of winged individuals produced was greatly reduced, even to zero, if winged parents were starved. Indeed, Ackerman reports a decrease of wing-production when starvation of winged females was obtained by removal from the plant. Ackerman concluded, on the evidence as a whole, that abundance of water in the food, that is, dilute sap, favours wingless forms, while more concentrated sap favours wings. This conclusion agrees well with the less specific one of Davidson (1914) that a change of sap causes wing-production, and with the observation by Davidson (1921 *a*) that deterioration of the plant brings on wings, and would explain why, on young plants, only wingless aphids should appear (Klodnitski, 1912).

#### *Chemical substances.*

The effect of substances in the medium is probably closely related to nutrition in a broad sense. It is particularly difficult to separate these agents in the case of aphids, where the substances must be administered to the host plant.

*Chemical factors in the rotifer cycle.* The earliest discovery of an effect of the chemical composition of the medium on the rotifer cycle was that of Shull (1910 *a*, 1910 *b*) who found that raising *Hydatina senta* in a fairly strong solution of horse

manure, the liquid in which the rotifers' food was being reared, caused the gamic females to disappear. An attempt was made later to discover what parts of the manure solution had this effect, and it was found (Shull, 1911) that urea and the ammonium salts had such an effect, though not as marked. Other chemical substances reducing the number of gamic females were sodium hydroxide, butyric acid, cane sugar, beef extract, creatin, potassium sulphate, ferric chloride, and bouillon. All had the effect of suppressing gamic females, except very dilute calcium chloride (Shull, 1913 a). That it was not merely the osmotic pressure of these solutions, not their acidity nor alkalinity, not merely their delay of physiological processes, that caused them to affect the cycle in like manner, was shown later by Shull and Ladoff (1916). In the meantime, Whitney (1910) discovered that a freshly made manure solution, if very dilute, increased gamic reproduction, and concluded that some transitory substance present in such a new culture must produce gamic females. In view of the work of Tauson (1925, 1926 a, 1927 b) and of Luntz (1926), soon to be described, it seems likely that the stimulating action of dilute fresh manure solution may be due, not to any particular substance in it, but merely to the change which it introduced.. Similarly, the exceedingly dilute solutions of ferric chloride and other substances with which Moro (1915) induced gamic reproduction in *Brachionus pala* may act merely as changes in the environment, not as specific agents.

In the midst of the above discoveries, Whitney made known the effect of green food, already described, in stimulating the production of gamic females. Since oxygen is liberated by such organisms during photosynthesis, Shull and Ladoff (1916) tested separately the effect of oxygen, and obtained an increase of gamic females, though not nearly so marked an increase as Whitney's *Chlamydomonas* produced. Oxygen was found particularly effective in counteracting such repressants as bouillon or manure solution. Later experiments by Shull (1918 a) indicated that when *Euglena* is used as the green food, from one-fourth to one-third of its total effect in increasing gamic reproduction, which was a little more than enough to counteract strong manure solution, might be attributed to the oxygen liberated, the balance to the food as food. A second strain of rotifers showed a distinctly smaller effect of the oxygen.

Whitney was unable to find the effect of oxygen claimed by Shull. Extra oxygen, administered when *Chlamydomonas* was being used as food, he found (Whitney, 1917 a) did not increase gamic reproduction, and he supposed that in Shull's experiments it had merely served to increase the protozoan food and thus increase gamic reproduction as a nutritive phenomenon. Whitney (1919) again concluded that oxygen was ineffective, since, with *Chlamydomonas* as food, fewer gamic females were produced in the light than in darkness, and he suggested that light caused the *Chlamydomonas* to attach to the glass where they could not be devoured, and that lowered nutrition caused fewer gamic females to develop. Watka (1928) was also unable to get gamic females by use of oxygen. However, Tauson (1927 b) listed oxygen as the third most effective of all agents, not of itself, but through changes in its concentration.

The recent work has dealt with pH of the medium and definitely known chemical content, with a refinement not approached in the earlier work. Tauson (1925), working with *Asplanchna intermedia*, showed that change of pH in either direction causes gametic reproduction, but that if the pH is then kept constant at the new level, the effect gradually disappears. The pH value he regarded as the most important of all external agents, and its effects are accentuated by temperature changes. Luntz (1926), working with *Pterodina elliptica*, got no effect of pH by itself, but found that at certain levels it inhibits the effect of change of food.

With respect to salt content of the water, Tauson (1925) reported that increase of carbonate decreases gametic reproduction, and *vice versa*, and later (1927 *b*) listed the carbonates as the fourth most powerful of external agents. Their effect is largely in relation to pH effects. Calcium has no effect by itself, but modifies the effect of pH. Certain other substances appear to have no effect of any sort. According to Luntz (1926), changes in the salt content (standard Benecke solutions were used) within a certain range induced, by themselves, gametic reproduction, while in other ranges they modified the effects of change of food (described in a previous section).

All this work indicating that change, merely as change, stimulates gametic reproduction is quite in keeping with the results of change of food, and recalls such observations as those of Kahn (1921) who found that changing rotifers from one natural water to another brought on the gametic forms. However, not all investigators have obtained results with chemical substances. Mitchell (1913) and Noyes (1922) report negative results from such experiments.

*Chemical substances and the cladocera.* The first direct application of chemical substances to the modification of the cycle of the daphnids seems to have been made by Schmankeiwitsch (1875), when he changed *Daphnia magna* from weak to stronger salt solutions and obtained males and fertilised eggs. Acidity and alkalinity were tested by Papanicolau (1910 *b*) without result. The next direct test was made by Green (1919) who applied sodium chloride to *Simocephalus vetulus*, without, however, obtaining any increase in gametic reproduction. Banta and Brown (1924 *a*, 1924 *b*) successfully employed chloretone, uric acid, and carbon dioxide to induce the production of males, and alcohol and adrenal cortex to suppress gametic forms. These appear to be the only substances directly used to control the cycle.

Supposed chemical changes have been obtained, however, in other ways. Kurz (1874) stated that he was able to produce intermediate forms of *Daphnia pulex* at will by evaporating the water they were in down to one-sixth or one-eighth of its original volume, by which time both sexes were always present. Green (1919) made a similar attempt, but without success. Crowding the daphnids, or allowing them to remain long in the same water, resulting presumably in an accumulation of their excretions, has been repeatedly shown to induce gametic reproduction. Langhans (1909) pointed out that these excretions reduce growth and reproduction, and since gametic forms appear at times of maximum numbers, the two phenomena probably stand in relation of cause and effect. Hastening of gametic reproduction by excretions was observed by McClendon (1910), Grosvenor and Smith (1913), G. Smith (1915) who stated that the effect of crowding is on the excretions, not

on the food, de la Vaulx (1922), and Banta and Brown (1923, 1924 *a*, 1924 *b*, 1928). Quite consonant with these results is the statement of Langhans (1909) that removal of the animals repeatedly to clean water prolongs parthenogenesis indefinitely. A similar view regarding clean water was expressed by Papanicolau (1910 *a*), but later (1910 *b*) he stated that excretions have not been shown to reduce anything but numbers. Hartmann (1919) included excretions as one of the factors causing depressions, which in his opinion makes them a probable cause of gamic reproduction. One race of *Daphnia pulex*, in the experiments of Banta (1925 *b*), reacted peculiarly to crowding, in that ephippia were produced, the eggs in which were, however, parthenogenetic, and that no males resulted from crowding in this race. Crowding is not always necessary to obtain gamic forms, however, for Agar (1914) changed the water frequently, and permitted no crowding, yet obtained males; moreover, these males would appear in the first broods of the families.

The nature of the excretions has been seldom discussed. Banta and Brown (1923) stated that one experiment suggested carbon dioxide (and depletion of oxygen), but it is likely that other substances are in the minds of most investigators. How the excretions work has been considered by several authors. Thus, G. Smith (1915) pointed out that, under crowding, the animals store fat, rather than glycogen, and that fat is characteristic of the males and gamic females. According to Banta and Brown (1924 *a*, 1924 *b*), substances which increase the rate of metabolism (alcohol, adrenal cortex) favour parthenogenesis, but that anything which reduces metabolism (crowding, carbon dioxide, uric acid, chloretone) may be expected to produce males. These males, however, have a higher rate of metabolism than the females, as in so many other animals; and the sexes respond to the Manoilov test (Banta and Satina, 1925) in the same manner as in strictly bisexual species.

*Chemical substances and the wings of aphids.* The work done on aphids by means of chemical substances all relates to wing-production, none to gamic reproduction. Clarke (1903) is said to have obtained many more wings in the rose aphid if it was reared on cuttings kept in sand moistened with strong solutions of magnesium chloride or sulphate than with solutions of potassium or sodium salts or distilled water. Neiils (1912) confirmed Clarke's results on the same species, though the numbers involved were small. Shinji (1918), using also the rose aphid, reported many winged individuals on cuttings treated with salts of the heavy metals or magnesium, or with sugar, and practically no wings with a number of other substances. Wadley (1923) obtained very small, perhaps not significant, increases in wing-production with various salts, and dismissed them as not important in nature, anyway. However, Mason (1922, 1923), using *Macrosiphum davisii*, found no effect of magnesium sulphate, and Haviland (1921), working with *Myzus ribis*, obtained fewer winged forms with magnesium sulphate than with tap water (which, unfortunately, was not analysed). Ackerman (1926) was also unable to obtain excess of wings by any of the chemical treatments employed.

In view of these highly contradictory results, it must be said that an effect of chemical substances has hardly been proven. This is the more likely since such substances must be administered, not directly, but through plants. Protoplasm is



highly selective in its absorption of salts, and some of the concentrations used in the experiments almost certainly killed it. No one has analysed any of the plants treated, to ascertain what, if any, change has been effected in them. Moreover, there are obviously so many factors affecting wings of aphids that the exclusion of all of them except the supposed chemical difference in any experiment involving a living plant seems rather unlikely. Indeed, the chemical difference may—even must—have produced other differences which may not have been chemical at all (differences in nutrition, for example).

### *Light.*

With two exceptions, Watka (1928) who experimented with rotifers without result and Hartmann (1921) who found strong light a factor in the depression periods of cladocera, all of the work with light in the three groups with which we deal has been done on aphids. Light was long considered an indirect agent, affecting the growth of the host plant and hence the nutrition of the aphids (Mordwilko, 1908). Probably the first suggestion that light might be a direct factor was made by Hunter (1911) who was studying the gamic forms in *Toxoptera graminum*, but the suggestion was only a statement of a programme for the future which seems not to have been carried out.

To Marcovitch (1923, 1924) belongs the credit of the discovery that light is an important factor in the cycle of the aphids. His conclusion was that a shortened day induced gamic reproduction and wings. Not all species acted in this way, however, and controls were lacking in some of the important experiments. Shull (1926, 1928) carried the work further with *Macrosiphum solanifolii*, with the following results. Wingless aphids reared in continuous light or continuous darkness produce almost all wingless offspring; but if the parents are alternated between light and darkness of certain durations, their offspring are nearly all winged. The effect of the light and darkness was proven to be directly on the aphids, not on their host plants. Removal from the plants for a certain length of time daily during light produced a moderate proportion of winged offspring, while removal from the plant during darkness had no such effect. In later work by Shull (1929) it has been shown that, for the shorter durations of light, the longer the light lasts and the more intense it is, the more winged offspring are produced. The maximum effect of alternating light and darkness was obtained by about 6 hours of light and 12–14 hours of darkness. The effect was gradually diminished if the period of light were made more or less than 6 hours and if the darkness were made more than 14 hours long; but the effect was very *suddenly* reduced if the periods of darkness were made less than 12 hours. The most sharply defined time element was this requirement that darkness should last at least 12 hours. Alternating light and darkness in various time ratios proved effective, with only small differences, provided the dark periods were 12 hours long.

The effects described were produced at temperatures up to 20° C., but rapidly disappeared at higher temperatures, until at 26° C. alternating light and darkness did not produce wings at all.

To explain these results Shull postulates a substance produced, perhaps photochemically, in the light, and converted into something else in darkness. The concentration of this latter something determines, he supposes, whether wings shall be produced.

*Physiological mechanism of response.*

The fundamental aim of the study of these cycles is discovery of the physiological processes involved in them. While knowledge of these processes has scarcely begun to exist, it is possible to point out the things most necessary to explain or most fruitful of evidence, the way of approach, and the steps already attempted along that road.

*Time of action of external agent.* One of the chief clues to the ultimate physiology of the cycles will no doubt be the time in the life of the individual in which critical events occur. With respect to the cladocera there is fair agreement. Woltereck (1911) concluded that whether an egg was to become a male or an ephippial egg was decided either just before it leaves the ovary, or at three much earlier times. Green (1919) and Banta and Brown (1923, 1924 *b*, 1928) limited the decision to one particular time, which the former stated to be before the egg leaves the ovary, the latter about four hours before the egg is laid. The latter statement is based on the fact that crowding is followed within four hours by the first males. In view of the difficulties of ascertaining more remote times, it seems likely that Woltereck's three earlier times may not exist, although the possibility of other susceptible periods must not be overlooked. Within the last few hours before it is laid, the egg undergoes its maturation division or divisions, which makes this seem an especially probable labile period.

In the rotifers, Shull (1912) showed that whether an egg becomes a gamic or a parthenogenetic female is decided within three hours before the egg is laid. Manure solution or bouillon of a concentration which completely excluded gamic females, was ineffective on eggs before they reached their final growth stage or after they were laid. The three hours before laying includes, as in the cladocera, the maturation process. Confirmatory conclusions were reached by Mitchell (1913) who stated that high nutrition must be applied to the mothers to induce gamic daughters in *Asplanchna*, by Moro (1915) whose chemical treatments of *Brachionus pala* were effective only on young individuals not yet born, and by Luntz (1926) who found that change of food just after one egg is laid causes the next egg of *Pterodina elliptica* to become a gamic female. Mitchell, however, concluded that starvation is also a factor in producing gamic females, and that it works on the oöcytes of the young female, to determine whether the oöcytes shall become gamic eggs. As pointed out above, and by Shull (1913 *b*), the ten individuals used by Mitchell in his tests seem scant evidence in an animal whose cyclical phenomena are as erratic as those of the rotifers. Hence it is not unreasonable to doubt the validity of Mitchell's latter conclusion. Storch, in his paper of 1923, implied that treatment of a young female might cause it to become gamic, by suggesting that anything which causes the chromosomes to enter synapsis (as they do in young gamic, but not in parthenogenetic females) would cause that individual to become

gamic. Apparently this suggestion is not repeated in his later paper (Storch, 1924). On the whole there seems to be as yet no necessity of supposing that there is any other labile period in the rotifers except that of the maturation of the egg.

In the aphids, the only evidence regarding the time of determination of gamic forms is the work of Shull mentioned above among the effects of temperature. If winged females are changed from low to high temperature, they change from a progeny consisting almost wholly of gamic females to one of almost all parthenogenetic females, in a period of 10 days to 2 weeks. The change is not a sudden, sharply defined one as in the rotifers, however, but is gradual, and intermediates are scattered along the latter third of this period. No calculation has yet been made of the stage which an aphid egg has reached two weeks before the offspring developing from it is born.

Concerning the determination of aphid wings there is little agreement. Neils (1912) and Shinji (1918) using chemical substances, and Wadley (1923) using temperature and crowding, all concluded that wings may be determined in the lifetime of the individual—Neils within 3 days after birth, Shinji 2–3 days after birth in one species and 5–7 days after birth in another species, and Wadley before the second moult—though there is room to conclude from Wadley's statements that starvation or crowding also worked on the parents to make the offspring winged. Shull (1928), on the contrary, showed that the whole effect of light on wing-production occurs within the last two days before birth. Moreover, as is pointed out more specifically below, there is a very strong tendency for winged parents to produce wingless offspring, even when the conditions are such that wingless parents produce many winged offspring. It must therefore be concluded that there is a very potent wing factor regularly acting before birth.

*A common basis of environmental action.* It is interesting, perhaps important, to note how many of the external agents which modify the cycles of these animals are effective only when they constitute a change from some previous condition. Thus, according to Luntz (1926), it is change of concentration of substances in the water, change of food, that induces gamic reproduction in rotifers. Tauson (1927 *b*) showed that change of pH, change of oxygen, and change of temperature cause gamic females to appear in the same group. Shull (1928) found that change of light and darkness induces wings in aphids, while Ackerman (1926) concludes that the effect of a change of temperature on the wings of aphids soon disappears under constant temperature. Whitney (1924) calls his use of *Chlamydomonas* a sudden change from *Polytoma* as food, and his conclusion (Whitney, 1910) that there is a transitory substance present in new dilute manure cultures which induces gamic females suggests that mere change was the real factor. Not improbably many of the other factors which have been found to modify the various cycles do so merely because they are changes from previous conditions. In experiments lasting only a short time, as many of those reported necessarily did owing to the short life of the animals employed, the gradual disappearance of an effect under the constant conditions following a change would not be witnessed. Not all environmental effects can be interpreted as due to change, but the number which could be is greater than

appears on the face of the evidence. Too little is known of the general physiological effects of a changing environment to pursue this matter further and suggest a fundamental explanation, but the subject offers an engaging clue for the future.

*The physiological mechanism.* Little is actually known of the intimate manner in which the protoplasm of these animals responds to environmental treatment. This section must therefore be either short or speculative. Issakowitsch (1905) devised a very simple scheme to account for the production of males by cladocera under reduced nutrition. It was simply that the available nutrition could not produce eggs of the size necessary to become females, hence males were produced. His explanation of the production of ephippial eggs upon still further reduction of nutrition did not include physiological details. McClendon (1910) suggested that the agents favouring ephippial eggs did so by reducing the permeability of the plasma membrane of the eggs, thus causing them to require the additional stimulus of fertilisation. Banta and Brown (1924 *a*, 1924 *b*, 1928), as has been stated above, held that decrease in the rate of metabolism induces the production of males. In rotifers, Storch (1923, 1924) observed certain highly refractive bodies situated at the nuclear membrane of oöcytes in the gamic but not in parthenogenetic females. What physiological processes they might signify can only be conjectured.

The most highly evolved physiological conception relating to any of these cycles is that of Ackerman (1926) applied to wing-production in aphids. The haemolymph of the aphids contains numerous globules of presumably a lipid substance. This substance differs physically, depending on the temperature at which the aphids are raised, so that in aphids raised at 28° C. these globules solidify at about 9° C., while in aphids raised at 12° C. the globules solidify at about - 2° C. Between these extremes there is a graduated series. In winged aphids, he found the solidification temperature of the globules to be always 1°-3° C. lower than in wingless aphids reared at the same temperature. When aphids were changed from one temperature to another, it required more than 14 days for the solidification temperature to drop to the degree characteristic of the new temperature. However, if the aphids were crowded, the solidification temperature changed about twice as fast as when they were not crowded. Young aphids born at a new temperature changed their lipid-solidification temperatures more rapidly than their parents did. These effects appeared to be on the aphids directly, not indirectly through the plants. This evidence is circumstantial, but Ackerman believed it indicated some important relation between lipid solidification and wing production, both, perhaps, being results of one cause.

Besides the colourless lipid globules, the aphid used by Ackerman possesses some brown globules which, when treated with an alkali, become red. During the early stages of wing development, the haemolymph in the region of the wing buds is often purplish and, on treatment with alkali, becomes red. Ackerman concludes from these facts that the brown globules are disrupted during wing development, and their contents dissolved in the haemolymph. Certain microchemical tests indicated that some of the material was absorbed by the large lipid globules, and it was found that the solidification point of the latter was thereby raised. Moreover;

lowering the temperature of an aphid was found to cause the disruption of all the brown globules. Add to this that adult winged aphids immersed in sodium hydroxide became much less red than did adult wingless ones so immersed, demonstrating a smaller quantity of the responsible substance in the winged aphids, and the connection between the brown globules and wing-production is circumstantially fairly complete.

Ackerman's theory is that the brown globules are disrupted by mechanical disturbances, or temperature, or perhaps chemical disturbances, and that their substance is dissolved in the haemolymph and its contained lipoid globules, causing changes in the solidification temperature of the latter. Some part of this series of events, he supposed, stimulates the development of wings. The theory appears to him to explain the effect of external conditions, why these external conditions affect winged aphids much less than wingless ones, and why winged aphids have a much smaller tendency to produce winged offspring than wingless aphids have.

#### *Internal factors.*

Though it is rarely possible to separate internal factors completely from external ones, there are some features of the cycle in these groups which are relatively independent of the environment.

*Age of parent.* Shull (1910 *a*) found for the rotifer *Hydatina senta* that gamic females appeared distinctly more often in the middle of their mothers' families than at either the beginning or the end. Luntz (1926), however, reported for *Pterodina elliptica* that the response to external factors was the same at all ages. For the aphid *Pemphigus spirothecae* Mordwilko (1907 *a*) stated that the first eggs are large and produce females, the later ones are small and produce males. In the cladocera, Papanicolau (1910 *a*) found that the early broods of females had a strong tendency to be parthenogenetic females, the late ones gamic forms, while the middle ones were more balanced and easily turned one way or the other by external agents. More recent workers appear not to confirm this conclusion of Papanicolau, or attribute it to a changing environment.

*Form of parent.* In aphids it has been repeatedly observed that winged parents have a much smaller tendency to produce winged offspring than wingless parents have (Klodnitski, 1912; Gregory, 1917; Shull, 1918 *b*; Davidson, 1921 *c*; Mason, 1922, 1923; Wadley, 1923; Ackerman, 1926; Reinhard, 1927). Almost an alternation of the winged type in one generation with wingless in the next, and so on, was reported by Mason, as a result of this factor. Also, Ackerman and Reinhard show that such wing-producing factors as crowding and starvation have a much smaller effect on winged parents than on wingless ones. According to Ackerman's theory, described above, the difference is due to a smaller amount of the substance contained in the brown globules (in his species) in the winged forms. Ewing (1926) assented to the general conclusion that wingless mothers tend to produce winged offspring, and earlier (Ewing, 1925) suggested that the effect is cumulative, since the tendency to produce winged offspring was greater after many successive generations of wingless ancestors. The latter suggestion seems now to be highly improbable.



In many, perhaps most, aphid species there is a strong tendency, when the gamic forms are produced, for females and males to be born of different types of parent. Usually the male is produced by a wingless mother, the female by a winged mother. Perhaps there is the same relation here as that stated above, that winged parents have a strong tendency to produce wingless offspring, and *vice versa*, since males are usually winged and gamic females wingless, though wings cannot be the only thing involved in their production. However, there are variations in different species, for Klodnitski (1912), in a criticism of Balbiani, states that both gamic females and males may come from one mother, and Baker and Turner (1916) apparently believe this to be quite common.

*An inherent cycle.* Ever since Weismann's (1879) work on daphnians, and his conclusion that the different species are adapted to the bodies of water in which they live by entering upon the gamic phase of their cycle frequently or not, according as they are likely to be required to endure drought at frequent intervals or not, a controversy has waged over the possible existence of inherent cycles in the cladocera. Weismann's idea was that gamic forms appeared every so many generations, a different number in different species, and that the length of the cycle had been arrived at by natural selection. Support for this idea came later from Keilhack (1906), who saw in the dicyclic condition of *Polyphemus pediculus* a reminiscence of former arctic winters and an adaptation (by a repetition of the original cycle) to our milder modern seasons. Issakowitsch (1905) had concluded that there was no cycle in Weismann's sense, since he believed nutrition to be in control of gamic reproduction, but Keilhack argued that a gamic phase in June could not be due to lack of nutrition, a contention supported by Strohl (1907, 1908). Issakowitsch (1908) admitted that there must be a cycle in the sense that a gamic phase must be gone through, but insisted that the number of generations in it is determined by external conditions. Von Scharfenberg (1914) stated that in *Daphnia pulex* there is a strong tendency for gamic forms to appear in certain generations, which does not exist in *Daphnia magna*, and had earlier concluded (von Scharfenberg, 1911) that the influence of food on the cycle is an inherited quality acquired through natural selection. Papanicolau (1910 *a*) regarded this latter idea as untenable, Agar (1914) said there is no certain number of generations in the cycle, and Banta (1914) concluded there is no inherent cycle; but van Herwerden (1918) accepted the innate cycle of Weismann.

If by cycles it is merely meant that different species or different strains respond in different degree to external conditions, by producing gamic forms earlier or later, or in greater or smaller numbers, probably most students of the cladocera now would admit that there are cycles. Woltereck (1909, 1911, 1928) points out that this is true, and suggests an internal system of "paralysators" and "activators" which become latent by turns, and upon which environmental factors may work. McClendon (1910) recognised an inherited factor, and Banta (1914 *et seq.*), who has repeatedly pronounced against a cycle, must accept the existence of inherited differences.

A graduated cycle in the cladocera was proposed by Papanicolau (1910 *a*) who

thought he saw plain evidence of an increasing gamic tendency from generation to generation. Agar (1914) mentioned a similar increasing tendency to produce gamic forms, but attributed it to the cumulative effect of environmental factors. Green (1919), on the contrary, found no such tendency in *Simocephalus vetulus*, and Banta (1925 a), after rearing 300 successive parthenogenetic generations of *Moina macrocopa*, found it was no easier then to call forth males by crowding than it had been in the early generations. Banta (1915) had also earlier shown that there was no progressive diminution of vigour after long-continued parthenogenesis, hence no inherent depression which Hartmann (1919) and others looked to as a cause of gamic reproduction.

An inherent cycle in the rotifers was assumed by Lauterborn (1898), who listed dicyclic and polycyclic species, and, though admitting the effects produced by temperature (Maupas, 1891) and by food (Nussbaum, 1897), concluded such effects could not be general. He regarded the cycle as regulated by internal factors, the gamic forms appearing in certain generations. Dieffenbach and Sachse (1911) also described species as monocyclic and dicyclic, and related the phenomenon to food, though whether physiologically or through natural selection is not wholly clear.

That strains differ from one another in their proportion of gamic forms under like conditions in the rotifer *Hydatina senta* was suggested by Punnett (1906) and shown by Shull (1911) who crossed and back-crossed such lines and obtained different percentages of gamic females in each case. Similar differences were observed by Whitney (1912) in the same species, and by Luntz (1926) who worked with two strains of *Pterodina elliptica*, one of which produced 96 per cent., the other 50 per cent., of gamic offspring under the same external conditions.

A gradual decrease in the percentage of gamic forms from generation to generation was observed by Whitney (1912) and by Shull (1912). The latter described the phenomenon as a result of long-continued parthenogenesis, without, however, intending to imply exactly that this mode of reproduction was responsible for the decline. His idea, as explained later (Shull, 1923), was that the decrease of gamic reproduction was due to long-continued undisturbed metabolism. Gamic reproduction, had it intervened, would have provided the disturbance necessary to increase the proportion of gamic forms in succeeding generations, though it is probable that such an increase would be found only in the lines derived from certain, not all, of the fertilised eggs. As it has turned out since, an even more effective disturbance of metabolism has been provided by change of food, change of chemistry of the medium, and change of temperature, as described in a previous section of this paper. Recent workers, employing these changes to induce gamic reproduction, have not observed its gradual decline through numerous parthenogenetic generations (Tauson, 1926 b; Luntz, 1926).

An inherent reproductive cycle, different in different species, was held to exist in aphids by Klodnitski (1912), who stated that up to that time there had been no proof of any modification of the cycle by external factors. Baker and Turner (1916) concluded that, after temperature, the most important factor in gamic reproduction in plant lice is the number of parthenogenetic generations that have elapsed since

the fertilised egg. They state, however, that successive generations appear to require more and more severe conditions to bring forth gametic forms, which is a reversal of what would be expected on the theory of an inherent cycle. Davis (1909), on the contrary, proved that gametic forms in several species do not appear in any definite generation. A progressive increase in the tendency of wingless females to produce gametic daughters in *Macrosiphum solanifolii* was observed one autumn by Shull (1918 b). It is usual for wingless females to produce males, and he has not witnessed their production of gametic females in such numbers since. The reason for this exceptional parentage one season is unknown.

In the same year in which the above observation was made, but during the summer, Shull (1918 b) also obtained a gradual increase in the number of winged individuals through a number of generations. In view of recent work (Shull, 1928) it seems likely that this change was due to some progressive change in the natural light conditions then prevailing, and that no cycle of wing-production was indicated. Wadley (1923) found no evidence of different strains or inherent factors in wing-development, but Davidson (1927 b), unable to account for observed phenomena by environmental factors alone, assumes an intrinsic factor as well. Perhaps the strongest evidence of inherited differences in wing-production is found in unpublished experiments of Shull demonstrating very striking differences in the response of two strains of the same species, *Macrosiphum solanifolii*, to alternating light and darkness, the results of which are, however, still too meagre to discuss further.

*Periodicity.* The periodic recurrence of gametic forms at intervals, especially noted in the rotifers, is probably closely related to the problem of cycles, but is doubtfully included here as due to internal factors. It must have been observed by every student of rotifers who has worked with several species, that gametic females and hence males tend to occur in "epidemics." Lange (1913-1914) regarded this as due to an internal rhythm, to which external factors are secondary. Mitchell (1913) described periods of rapid multiplication, which nearly coincided with periods of gametic reproduction, and considered the former to be the cause of the latter. To Shull (1913 b), however, the two events seemed rather to be two results of a single cause, a view which finds support in the work of Wesenberg-Lund (1923) who, after pointing out that increase of numbers precedes gametic reproduction, went on to show that maxima of numbers are sometimes passed through without the appearance of gametic forms. Such epidemics of gametic reproduction, if they occur at irregular intervals, could well be explained by occasional changes of food or the composition of the water. However, Shull (1915) observed in one strain a recurrence of gametic forms every four weeks, and in another strain every nine weeks, with considerable regularity. Luntz (1926), finding no evidence of such periodicity in *Pterodina elliptica*, concluded that Shull must have changed the water or something every four weeks. That can hardly be the explanation, since the water was purchased from a commercial concern in large enough quantities to last six months to a year, and the quality of one shipment was presumably uniform, while food cultures were changed every several days. Moreover, the occurrence of a 4-week

and a 9-week periodicity at the same time seems to preclude Luntz's explanation. It seems not impossible that gamic reproduction is commenced when a certain substance is present in a given concentration in the protoplasm, that this substance is exhausted in the production of the gamic forms, and that a repetition of gamic reproduction must await the re-accumulation of the required amount. Under changing conditions of food or chemistry of the medium, such re-accumulation may be supposed to proceed rapidly, and every generation might include many gamic forms; but under uniform conditions, it would presumably occur slowly. At the basis of this physiological process would no doubt be a genetic factor which would cause one line to require four weeks, another nine weeks, and so on, to reach the threshold of stimulation.

In aphids, Davidson (1924) concluded that there is a periodic rhythm of gamic reproduction, though an elastic one; and in cladocera such cyclical phenomena would be explained by Woltereck (1911) by competing substances, of which now one, now the other would gain the upper hand.

*Intermediates.* The occurrence of individuals combining certain characteristics of two types or intermediate between them has often been reported for the cladocera and the aphids. In cladocera, they are mostly intermediates between males and females. Kurz (1874) described such forms for three species, and called them hermaphrodites. He was able to produce them at will by evaporating the water down to a fraction of its original volume. Intermediates are mentioned by Kuttner (1909), and are described in detail by de la Vaulx (1915 *a*, 1915 *b*) who appends a list of the literature concerning them. Certain lines, no doubt originating from mutations, have produced many such forms in the work of Banta (1916 *a*, 1916 *b*, 1916 *c*, 1917, 1918). One line was of the species *Simocephalus vetulus*, and included individuals combining in every conceivable way the primary and secondary differences between the sexes, some being hermaphrodites in the sense that both eggs and spermatozoa were produced. This line has four times branched off normal lines, presumably by return mutations, during the time it has been reared. Several similar intermediate-producing strains have arisen in *Daphnia longispina* (Banta, 1918), but none in four other species. De la Vaulx (1921) recorded 350 intermediates in a line of *Daphnia atkinsoni*, and showed that the tendency to produce them is hereditary.

Intermediates between gamic and parthenogenetic females have been described in *Daphnia pulex* by Banta (1925 *b*) under the name of pseudosexual females. These produce ephippia, the eggs in which develop parthenogenetically. All of these occurred in one line, and no males were produced in that line. Probably a similar interpretation can be put on the observation of Olafsson (1918) that ephippial eggs develop parthenogenetically in the cold waters of Spitzbergen (cited by Woltereck, 1928).

Intermediate aphids appear to be fairly common. Moritz (1893) described a pupa with only folds of the skin for wing pads, and other intermediates between winged and wingless forms were mentioned by Mordwilko (1909) and by Baker and Turner (1916). Klodnitski (1912) attributed them to the checking of wing-development by fresh food, and a similar explanation might be offered for those observed

by Turner and Baker (1915) who obtained them on young apple seedlings. More liquid food, such as would be secured from fresh young plants, was suggested by Ackerman (1926) as the cause of winged-wingless intermediates. Shull, in experiments not yet published, has obtained numerous intermediates in one strain, few in another of the same species, showing that there is also a genetic factor in their production. Intermediates between gamic and parthenogenetic female aphids were described by Hunter (1910), and have been artificially obtained in numbers by changing winged parthenogenetic females from low to high temperatures in unpublished work of Shull. Fatio (1876) is said to have observed a pupal *Phylloxera* laying gamic eggs which Baker and Turner (1916) thought must have been an intermediate, though all that appears to be shown is that it was paedogenetic. Others that were intermediate and paedogenetic were recorded by Ewing (1916). The laying of non-black eggs by virgin gamic females observed by Baker and Turner (1916) in the green apple aphid, and known to occur in other species, perhaps as a response to environmental conditions, may also be looked upon as intermediacy between gamic and parthenogenetic females, since the latter regularly deposit their reproductive products, while the former usually do not unless impregnated.

The occurrence of intermediates in these groups indicates that type-determination either is in a more or less fluid state, or that it consists of several events spread over a considerable time. No attempt appears to have been made to discover which of these possibilities is correct, as it would seem possible to do where artificial means of producing intermediates have been discovered. No one has reported intermediate rotifers, which probably means that the event which decides the difference between parthenogenetic and gamic females is a single sharply-defined one occurring in a very brief interval of time.

*Kernplasmarelation.* Applying Hertwig's idea that the volume of the nucleus relative to that of the cytoplasm is of importance in physiological processes, including sex determination, Issakowitsch (1907) sought to explain the cladoceran cycle by supposing that the ratio  $K/P$  (that of volume of nucleus to volume of cytoplasm) rises in case of degeneration or under those conditions which bring on gamic reproduction, and that the change of this ratio is the cause of the cyclical change. He reiterated this conclusion a year later (Issakowitsch, 1908). Papanicolau (1910 b) supported this claim by camera lucida drawings of intestinal cells from animals raised under different conditions. These drawings were later measured by Shull (1922) and in all cases but one showed differences of the kind which Papanicolau indicated, though one of the differences was small. The theory that relative nuclear volume is of importance in the cladoceran cycle was combated by Strohl (1907, 1908), von Scharfenberg (1911), and Woltereck (1911), but was championed by Hartmann (1919). The last-named author, from an examination of animals collected in nature, concluded that low temperature and long-continued parthenogenesis increased the ratio  $K/P$ , and that chemical substances also altered the ratio. The evidence of change was presented in drawings, not measurements, hence the numerical value of the increase is unknown. Not only the ratio of nucleus to cyto-



plasm, but that of nucleolus to nucleus, and of cell surface to nuclear volume, Hartmann regarded as significant for the cycle.

The only examination of the Kernplasmarelation of rotifers was made by Shull (1922) who was induced to test the question because of the superior features of the rotifer *Hydatina senta* for this purpose. It had been shown that chemical substances could exclude gamic females in this species, that gamic females occurred in epidemics in certain strains, and that more gamic females were produced in the middle of a family than near either end. Three independent ways of testing the nucleoplasm ratio were therefore available, and if that ratio were a causal agent it should undergo corresponding changes in all three circumstances. If it were merely incidental, it might coincide with expectation in one or more cases, but not in the others; or it might not fit the expectations at all. Shull examined the intestine, yolk gland and the oocytes, and though changes were found to take place in the *K/P* of some of them under some conditions, none of them was what would have been expected on the theory. In *Hydatina senta*, therefore, the Kernplasma-relation appears to have nothing to do with the cycle of reproduction.

#### *Chromosomes and maturation.*

There is general agreement now that in all three groups with which we deal the parthenogenetic eggs undergo only one division in maturation, while the gamic eggs divide twice, and that reduction of the chromosomes occurs in the latter, not in the former. Concerning the chromosome numbers, some details of maturation, and maturation in the male there are differences of opinion and differences in species.

In the rotifer *Hydatina senta* Lenssen (1898 a, 1898 b) found the diploid number of chromosomes to be 10 or 12, the two numbers probably representing uncertainty of count, not a difference between individuals, while 5 chromosomes were found in the mature gamic eggs. Whitney (1909), upon admittedly uncertain counts, thought the diploid number was 20-30, the haploid number in the gamic eggs 11-14, while Shull (1921) definitely concluded that the parthenogenetic egg received 12 chromosomes, the gamic egg 6, when mature. In *Asplanchna priodonta*, Storch (1923, 1924) observed synapsis, and the omission of a resting stage following the last oögonial division, in gamic females but not in parthenogenetic ones. Eight tetrads are formed, and two divisions occur in the gamic eggs, and 8 chromosomes are present at the end of maturation. In the mature parthenogenetic egg there are 16 chromosomes. Lehmensick (1926), in a study devoted mostly to other features, found 14-16 chromosomes in cleavage in this species. In *Asplanchna intermedia* there is an important difference of opinion. Tauson (1924) found 24 chromosomes in the parthenogenetic egg, 12 in the gamic egg, when mature; while Whitney (1924) counted 52 and 26 chromosomes respectively. Tauson, however, counted the diploid number (24) in the cleavage stages of the unfertilised gamic egg, so that the male possesses 24 chromosomes like the female. A doubling of the chromosomes must occur at an early cleavage stage of the male, or before. In *Asplanchna amphora*, according to Whitney (1929), there are 26 chromosomes in the mature parthenogenetic egg and the somatic cells of the embryo derived from

it. The chromosomes are larger in some such embryos than in others, and Whitney suggests the possibility that these two kinds are the parthenogenetic and gametic type of female. It is known, as shown in an earlier section, that at least in some rotifers the two kinds of female are already determined at a stage earlier than cleavage. Whitney found that the number of chromosomes in the mature gametic egg of this species is 13, and the same number occurs in the somatic cells of the embryo (the male) derived from an unfertilised gametic egg. In *Brachionus pala*, 10 chromosomes were counted (Marinelli, 1925) in mature parthenogenetic eggs; and while the reduced number was not actually counted in the gametic eggs, Marinelli conceded that in general male rotifers are haploid.

Spermatogenesis in rotifers has been carefully studied by only two investigators. Whitney (1917 *b*, 1918) had found that males of 11 species produce spermatozoa of two kinds: active functional ones, and smaller, rudimentary, motionless ones. In *Asplanchna intermedia* he concluded later (Whitney, 1924) that the primary spermatocytes, containing as he believed 26 chromosomes, do not usually divide into secondary spermatocytes, but transform directly into spermatozoa, which therefore have 26 chromosomes. Some of the primary spermatocytes, however, divide, each daughter cell receiving 13 chromosomes, and these cells he holds become the rudimentary spermatozoa. Tauson (1927 *a*), however, gave the following account of spermatogenesis in *Asplanchna intermedia*. Starting with 24 chromosomes, which according to Tauson is the diploid number for this species, the number is reduced to 12 in early spermatogonial divisions. There is only one maturation division, each cell receiving 12 chromosomes. These secondary spermatocytes are transformed directly into spermatozoa, all of which are functional. The account of spermatogenesis in *Asplanchna amphora* given by Whitney (1929) agrees with that of Tauson for *A. intermedia* in that only one division regularly occurs, the secondary spermatocytes receiving 13 chromosomes and becoming spermatozoa. Some secondary spermatocytes divide, however, each spermatid receiving fewer than 13 chromosomes. These spermatids, according to Whitney, become the rudimentary, non-motile spermatozoa.

The accounts of spermatogenesis in the rotifers are thus sufficiently contradictory in important respects, and so remarkable as compared with normal spermatogenesis, that it seems necessary to withhold a definite opinion regarding them until further critical examination of them is made.

Less has been done with the chromosomes of the cladocera. Kühn (1908) observed only one division and no reduction in the parthenogenetic eggs of *Daphnia pulex* and *Polyphemus pediculus*. The number of chromosomes was found to be probably 8. Schrader (1925) studied cytologically the pseudosexual or intermediate race of *Daphnia pulex* which Banta (1925 *b*) discovered, and counted 24 chromosomes, and observed only one division, precisely as in the normal parthenogenetic egg.

Spermatogenesis in *Simocephalus vetulus* was found by Chambers (1913) to involve two divisions, and a reduction of the chromosomes to 8 from a much larger undetermined number. About half of the spermatids were said to degenerate.

There was no observable difference between the degenerating and normal ones. In *Daphnia pulex*, Taylor (1914) counted 8-10 chromosomes in the spermatogonia, observed a well-marked synizesis in the spermatocytes, saw two divisions, and found 4 or 5 chromosomes in the spermatids. None of the spermatids appeared to degenerate. The male with its original 8-10 chromosomes thus appears to be diploid. In agreement with this evidence of diploidy in the males is the genetic evidence advanced by Banta and Wood (1928), who crossed a mutant short-beaked male with a normal wild-type female, and obtained some short-beaked and some normal wild-type offspring in every progeny. It would be possible, however, to explain this divided progeny by irregular dominance.

Of the aphids, *Aphis rosae* appears to be the first for which chromosome counts were made. Stschelkanovzew (1904) observed only one division in the maturation of the parthenogenetic egg, saw no early splitting of the chromosomes (which presumably has reference to synapsis), and counted 14 chromosomes. The number was changed to 10 for this species by Stevens (1905), Hewitt (1906), and von Baehr (1909), Hewitt suggesting that Stschelkanovzew counted cut chromosomes. Stevens showed further that these 10 chromosomes are of five different sizes, and that in the gamic egg, whose maturation involves two divisions, the final 5 chromosomes are of the same five sizes. In the parthenogenetic eggs, she at first saw no difference between those that were to develop into males and those that would become females. T. H. Morgan (1908, 1909), however, demonstrated a decrease of the number of chromosomes in the male-producing egg of *Phylloxera fallax* from 12 to 10, through the elimination of two whole chromosomes into the polar body; and von Baehr (1908, 1909) found a decrease from 6 to 5 in the male-producing egg of *Aphis saliceti* by a similar elimination of one undivided chromosome. Numbers of chromosomes in other species of aphids were reported by von Baehr as follows: in *Schizoneura lanigera*, 12; and in *Pemphigus pyrifomis*, 20.

The spermatogenesis of the aphids is illustrated by that of *Phylloxera fallax* (T. H. Morgan, 1908, 1909), in which the female has 12 chromosomes, the male 10. Eight of the 10 chromosomes pair, and separate at the first division, the other two going undivided to one pole. One cell thus receives 6 chromosomes, the other only 4; the former cell is much larger than the latter. The large cell divides into spermatids with 6 chromosomes each, while the small cell does not divide, but degenerates. A similar process occurs in *Aphis saliceti* according to von Baehr (1908, 1909), in which the female has 6, the male 5 chromosomes. The large cell produced by the first division in the male receives 3 chromosomes, and divides again into spermatids of 3 chromosomes each. The small cell resulting from the first division does not divide again, though it sometimes starts to do so before degenerating. As is pointed out below the functional spermatozoa are all female-producing.

#### Sex determination.

The expression "sex determination" is not here intended to be applied to the introduction of gamic forms, although the name has often been given to that.

phenomenon. Nor will any defence of the practice of calling the production of males and gamic females by the name "sex determination" be made, except to point out that it is more or less justified in the rotifers where the only way to obtain males is first to produce a gamic female, which is not true of the other groups. Determination of a gamic female rotifer is the last possible step in the determination of males, except one, namely, the prevention of fertilisation of her eggs. What is referred to as sex determination in this section is the *final* event which determines sex in any case.

In all three groups, fertilisation of a gamic egg is one such final event. All fertilised eggs of rotifers, aphids and cladocera become females. By analogy with other animals, it was to be expected that all functional spermatozoa would be found to be of the female-producing type. What evidence there is is mostly in support of this expectation, or is at least readily interpretable as support. In the aphids, as shown by T. H. Morgan (1908, 1909) and von Baehr (1908, 1909), two kinds of spermatids are produced, of which one degenerates. From the chromosome content of these degenerating cells, it must be inferred that they are the male-producing ones. In the cladocera Chambers (1913) found half the spermatids degenerating, and though he saw no morphological differences between the degenerating cells and the functional ones, suggested that the former were probably male-determining. Taylor (1914), it will be recalled, saw no evidence of any degeneration, but this is hardly surprising in such difficult material. The degenerating spermatids of aphids were overlooked for a time by competent cytologists who were deliberately searching for X-chromosomes. It may be confidently predicted that, when cladoceran spermatogenesis is better known, a mechanism comparable with that of the aphids will be found. In rotifers, Whitney (1917 *b*, 1918) discovered rudimentary spermatozoa making up about one-third of the total number. From this ratio he inferred that maturation in the male rotifer might consist of a first division in which unequal cells were produced, and a second division involving only the larger of the two cells. The small cell produced by the first division he assumed would have been of the male-producing type. The cytological evidence in its present state does not coincide exactly with this inference. In *Asplanchna intermedia*, according to Whitney (1924) as described above, the gamic egg has 26 chromosomes, the functional spermatozoa 26, while the degenerate spermatids (what few of them are produced) have 13. If one of the latter should fertilise an egg, there would be 39 chromosomes in the zygote, and there might be some question as to the sex of the rotifer developing from it. If spermatogenesis is correctly outlined by Whitney, one must probably infer that the number of chromosomes in both gamic eggs and functional spermatozoa has been at some time doubled, and that it was formerly 13. It is difficult, however, to find in the account of spermatogenesis in the same species by Tauson (1927 *a*) a ready parallelism with sex determination by two types of spermatozoa. It seems likely that there are still important discoveries to be made in the cytology of the rotifers.

Another important event which constitutes a final determination of sex occurs in the maturation of the parthenogenetic eggs of aphids and phylloxerans, as

discovered by Morgan and von Baehr (see above). The elimination of entire chromosomes into the polar body, by a division in which all other chromosomes divide, results in an egg with a slightly smaller number of chromosomes than in an egg in whose maturation all chromosomes divide. The chromosomes whose behaviour differs in different oöcytes are undoubtedly the X-chromosomes. However, too much stress must not be laid on this chromosome behaviour as a sex-determining process, since in the *Phylloxera* the eggs are small, and hence destined to become males, before the chromosomes are eliminated. Nothing comparable to this is known in the cladocera, since the male appears, from the available evidence, to have the same number of chromosomes as the female; and in the rotifers, the descent of the male from the gamic female throws responsibility for male determination back into the maturation of the egg from which the gamic female develops. What happens there is not known, but appears to have nothing to do with the number of chromosomes.

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# ANIMAL LANGUAGE IN ITS RELATION TO THAT OF MAN

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(Received March 18th, 1929.)

THE problem of the existence and characteristics of animal language has always exercised a certain fascination on the mind of the layman as well as on that of the scientist. In legend and fairy tale, in the belief of primitive peoples as well as in the stories of our children's books the animal able to speak a language that can be understood by man appears again and again. Popular belief credits most animals with the faculty of communicating their wishes, thoughts and feelings, at least to their congeners. Modern animal psychology has approached the problem of the language of animals in a scientific way and has studied it by careful observation, cautious interpretation and critical experiment and once again it was proved that popular and scientific opinion do not always agree. So it seemed desirable to bring together what at the moment is scientifically acceptable on this point, and to consider animal language especially as far as it corresponds with or differs from that of man.

If for a moment we imagine what happens between two human beings speaking together, it will be clear that for the purpose of communicating something to each other they produce continually varying combinations of *words*, i.e. special sounds, which show four characteristics: *first* that they are produced by the mouth and adjoining parts of the body; *secondly* that they are articulate, that is, built up out of one or more separate syllables, in contrast to the uninterrupted sounds man may produce in screaming or crying; *thirdly* that they have obtained a special meaning in the intercourse of man with man, a meaning that neither the speaker nor the listener knew at the beginning of his life but that has been learnt by him in intercourse with other men; and *fourthly* that these words indicate something, an object, an act, a concept, a state of mind, and so on. In other words, when we take human language as the prototype of real language, we may say that such a real language shows six characteristics: the sounds used in it are *vocal*, *articulate*, and have some *conventional meaning*, they *indicate* something, are uttered with the *intention* of communicating something to somebody else, and are *joined* together continually to form new combinations, so that phrases of various and different content are formed. Only when these six characteristics are present may we speak of a real language<sup>1</sup>. Now first of all we may ask if such a real language naturally exists in animals, and, if not, in what respect and to what degree the language of animals is deficient in one or more of these characteristics. Where this is the case we may follow Boutan<sup>(2)</sup> and speak of a "pseudo-language" of higher or lower degree.

<sup>1</sup> That even in man not all language is real language is a question that does not concern us for the moment.



Let us first consider the *technical* side of animal language and ask ourselves which animals possess *vocal*<sup>1</sup> speech.

It is a well-known fact that the great majority of animals are mute, and therefore unable to produce a real language. Thus all invertebrates are mute, with the exception of a few insects. But even these have no real voices, but produce their sounds in a different way<sup>2</sup>. In some grasshoppers the hind legs act as sound-producing instruments, while others make sounds by rubbing together the femur and tibia of one of the legs with one of the wings. *Mantis religiosa* is said to produce a sound by rubbing the extremity of the body against the wings, while crickets do the same by rapid vibrations of the tegmina or front wings. In the same way some butterflies (especially Arctiidae) in flying make a sound by the friction of the front legs against special parts of the wings; some ants are said to produce a sound by striking their heads against the leaves of which the nest is made; while the "death-watch beetle" (*Anobium*) makes its sound by striking its head rapidly against wood. And even the noisiest of all insects, the males of the cicadas, do not produce their sounds vocally, but by vibrations of the membranes on the ventral side of the body.

And even among vertebrates the greater number produce no sounds. Fishes are generally mute, and the relatively small number of them that produce some primitive sounds do not do so with the mouth but by other regions of the body (scraping of parts of the skeleton or movements of the air-bladder). Among the Amphibia the salamanders are silent animals and it is only in the males of the Anura that real voices occur. In Reptilia also the development of voices does not go very far: tortoises are silent, the sounds of the snakes are not vocal, for instance the rattling of the rattlesnake is performed by shaking the tail and their hissing is done not by the mouth but the nose. Vocal sounds in reptiles only occur in crocodiles and lizards (geckos). And so it is only among birds and mammals that real voices occur generally. But even here there are voiceless creatures. Several birds are only heard during the mating season, and according to Heinroth (11) the females of the nandu (*Rhea*) and the African ostrich (*Struthio*) are quite mute. Among the mammals this is the case with whales and dolphins, while giraffes too are said to be dumb. In this way the greater part of all animals lack the natural capacity of real language either because they are dumb or because they produce sounds not made in the mouth.

A second characteristic of real language is that it is *articulate*, that is to say, composed of syllables which are joined together into words. That such articulation is impossible when the sounds are produced by other organs than the mouth is

<sup>1</sup> In this article we are not concerned with the sounds that are produced passively and possibly unnoticed by the animal itself when walking, flying or swimming, as these sounds of course have no relation to any form of language.

<sup>2</sup> A few years ago Mangold (16) claimed to have observed sound-production in the common earthworm *Lumbricus terrestris*. From a terrarium in which some thirty to forty worms were living, sounds like "de," "di" or "da" were heard. Mangold believes that these sounds were produced by the animals, and that by movements of their tongues, that is the two lateral thickenings of the wall of the pharynx. If so the earthworm would not only be the lowest sound-producing animal, but even the only lower animal with a real voice. Mangold's observations however want further confirmation.

clear: the primitive way in which such sounds are formed by moving or rubbing together parts of the body does not allow of separate sound-units being formed. It is only the complicated muscular apparatus of mouth and tongue that makes possible the building and limiting of sounds to syllables. But do animals that have voices at their disposal form articulate sounds? Certainly the higher species at least would be able to do so, but yet as a rule they do not. It would seem that an exception is found in the sounds of some birds, as *e.g.* the "toc-toc-toc" of the cackling hen or the "cock-a-doodle-doo" of the crowing cock. But in the first case we have no true articulation, but rather a repetition of one simple sound. And as for the second case, the articulation of the sound is really not so sharp as is supposed. Bastian Schmid (19, 20, 21), in a careful examination of animal voices by means of objective methods, came to the conclusion that in most sounds of birds there is no real separation of syllables. And even when we admit that such is the case in the crowing of a cock, yet here too no real words are formed, that is, such syllables are not combined in different ways at different times, but are uttered only in the stereotyped way of the sound-utterance of the species.

More important however than these technical differences between animal and human language is what I would call the "sociological" difference between them. We have seen that it is a peculiarity of human language that the sounds used in it have a special *meaning*, a meaning that is not naturally known to the individual from birth, but has been learnt by him in the course of his life in intercourse with his fellow-men. It is especially this conventional meaning, conventional because generally there is no direct relation between the meaning and the nature of the sound, that turns an articulate sound into a word. With the aid of this conventional meaning it becomes possible to indicate something: an object, a situation, an act. Have the vocal expressions of animals such conventional meanings, formed by tradition and learnt by the individual through imitation? Or have they one innate meaning once for all, for all the members of the species, quite apart from intercourse with their kind? And, in either case, do these sounds indicate anything more or less concrete like the words of man do? In other words, may we say that the animal has real *words*?

That here again there exists an important difference between animal and human language, and that even with higher animals the sounds are innate and have no conventional meaning, was clearly shown in experiments made by Boutan<sup>(2)</sup> with the gibbon. Of all mammals the gibbon probably shows the greatest wealth of sounds, and as he belongs to the animals nearest to man the possibility exists that with him the sounds, or at least some of them, might be acquired from his fellows and might perhaps have a conventional meaning. Boutan separated a young gibbon from its earliest days from all other animals of the same species and kept it confined for five years, care being taken that during this time the animal never heard any sound produced by the gibbon in its wild state. Yet when the animal was adult it produced all the sounds of the species, as if it had continually lived among its congeners. Boutan therefore rightly concludes that the sounds of the gibbon are innate and are not acquired by imitation from other gibbons. And as in all circumstances

the isolated animal expressed itself in the same way as his congeners in the wood, it is clear that the sounds have no secondarily acquired conventional meanings but that their meaning is innate and specific. Further, it was clear to Boutan that these sounds do not sharply indicate objects or situations, but only seem vaguely to express some special general sentiments like fear or well-being. The gibbon therefore would seem to have no words, and that for two reasons: firstly his sounds have only an innate meaning, and secondly they do not indicate objects but express feelings.

With this conclusion Boutan comes into conflict with two other authors, who studied animal language, viz. Garner<sup>(8)</sup> and Yerkes<sup>(27)</sup>, who both credit monkeys with real words.

Garner particularly had a high opinion of the language of monkeys. Thus he believed that the capuchins have a special word for "monkey" as contrasted to other animals, and not only a word for "food" but even by slight modifications words for "bread," "apple" and "banana." According to him they even have words for "danger," for "friendship" or "love," and a shaking of the head together with a special sound meant a negation as in man. He therefore believed them capable of having long conversations together. With regard to the last-named assertion it is of importance to note that Garner made his observations chiefly on *Cebus* monkeys, probably the most talkative among primates, which, by their nearly continuous sound utterance may on the uncritical mind make the impression of telling stories to one another. That Garner so often describes these "stories" as being "sad ones" is a mere anthropomorphism, as he wrongly concludes, from the apparently plaintive voices of these monkeys, a sorrowful state of mind in them.

To tell the truth, Garner hardly justified his high opinion of the speech of monkeys. He even rightly remarked that it was difficult to find a word of human language that corresponded with one of the "words" of any of his animals. A sound that was supposed to mean "milk" or "food" really seemed to have a larger meaning, like "all that was desirable or agreeable". And his other examples also seem rather to express general feelings, like fright or hunger and the like, than to indicate special objects or concepts.

As for the origin of these words Garner believed that they are not always innate, but may be adopted by one monkey from another. Thus he reports once to have observed that a brown capuchin adopted two words from a *Cebus leucogenys*, with which it had lived together in a common cage for four years, and also the opposite case of a *leucogenys* adopting the word for "food" from a *capucinus*. He even describes how the white-cheeked monkey studied pronouncing the word and finally mastered it in about six weeks. Yet he admits that this is an exception, and that generally no imitation of their fellows takes place; in other words, that the meaning of their "words" is innate. If the case cited were true, we should here have a real word in animal language, i.e. a sound with a special acquired meaning, indicating a concrete object. But it seems more probable that what he took for imitation was an element of vocal expression common to two nearly related species and acquired somewhat late by the second monkey.

But it is not necessary to attach too much value to the assertions of Garner, which are not always very critical. More important is the opinion of Yerkes, who together with Mrs Learned recently studied the language of the young chimpanzee (27).

Let us state at once that Yerkes, more critical than Garner, recognises that, although vocal reactions in the chimpanzee are frequent and varied, real speech in the human sense is absent. Yet he believes that chimpanzees have special sounds to denote special situations or objects. And Mrs Learned, who made a particular study of these "words," in her summary gives a list of thirty-two of them. However, when we look somewhat more closely at these "words" and the situations in which they are uttered, they all seem rather to express general affects and feelings than to indicate special objects or situations. So for instance the sound "gak" or "gahk" or "gha," described by the author as a "food-word," seems to me rather to express the feeling of satisfaction when getting food or milk, and it seems rather bold to call this "the root word for food in the chimpanzee language," as Mrs Learned does (p. 63). For the rest, the same emotion may be expressed by "ko-ko-ko" or "ku-ku-ku," while "kah-hah" or "kuh-huh" seem to express the feeling of well-being, described by the authors as "laughter." So it seems that the authors have somewhat overestimated the value of the chimpanzees' sounds by calling them "words." And even if, with the authors, we could find "fruit motives" among the sounds of the animal, even then these sounds would not be words in the true sense, because their meaning was not acquired during a lifetime spent in intercourse with other animals, but was innate, and would therefore miss the characteristic of having a conventional meaning alone.

So I believe even Yerkes cannot convince us that monkeys possess words in their sound utterance. And *a fortiori* this must be true for the lower animals in their natural language, as for instance with cats. Mrs Gates (9) mentions an article of a Mr Grimaldi, who believes the cat has a language with six hundred fundamental words. It does not seem necessary to go much further into this contention.

So here again we find a typical difference between human and animal language. The former possesses words, the latter does not. The sounds in animal language have no conventional meanings; what might be called their meaning is something innate and typical to the whole species. Their sounds do not indicate objects or situations, but express sentiments and emotions, felt more or less clearly.

We have found as a psychological characteristic of human language that the words are uttered with the *intention* of communicating something to somebody. Can we admit the same for animal language? The layman will generally answer this question in the affirmative. He believes that animals really desire to communicate something to each other when they utter their sounds, just as is the case with man. However, a more critical observation makes it clear that, in this respect also, animal language shows an essential difference from that of man, and that animal sounds are generally uttered without any reference to other beings. We might express this by saying that, while human language in its essence is *allocentric*, animal language is naturally *egocentric*.

We have seen already that this was not the opinion of Garner. Garner positively declares that the sounds of monkeys are produced voluntarily and with premeditation; that they are always directed to special individuals with the clear intention of making the animal understood by its fellows. He believes monkeys have opinions on things, and try to express these by sounds. When they have completed their exposition they make a pause and wait for an answer, and when this answer is not forthcoming they repeat the same sounds as before. In the same way the cow, the sheep, and the goat have at their disposal the means to make themselves understood by their fellows. Their sounds are used in the same way as with us, viz. to give expression to thoughts.

Notwithstanding this explicit expression of the opposite by Garner I believe that there is no reason to assume that in its natural state an animal often wishes to communicate something to its fellow. This may certainly be said of solitary animals, to whom the existence even of animals of their own species is of no vital importance. The lion does not roar to warn other lions of his presence or to call the female, nor does the bird cry to claim its right on a certain territory, as is often found in popular books. When they roar or cry, they do this as an expression of well-being or self-assertion, without regard to the possible presence of other animals. And if there are other animals in the neighbourhood, which on hearing these cries are frightened away or attracted (if they are of the opposite sex), these are only secondary effects of the cries, never intended by the crying animals themselves. The primarily egocentric sound-utterance may in this way *secondarily* obtain the value of a means of communication; but that does not compel us to admit that this was the purpose of the crying animal.

The same holds good for animals living in temporary or permanent herds, although for them communication with their fellows may be supposed to be of more vital importance. When one of the members of a herd is frightened by some noise or sight and utters the "danger cry," this sound in itself is only a more or less involuntary expression of some strong emotion. Still it may secondarily obtain the significance of communicating the presence of some danger by the fact that, instinctively or by experience, it is understood by the other members of the herd as announcing danger.

And I even believe this to be true in cases where a strong social instinct might suggest a different view. I refer to cases where the mother animals "warn" their young in case of danger. It is often supposed that the mother hen for instance utters her danger cry with the clear intention of warning her chickens and calling them to her. I do not believe there is sufficient reason to admit this. A more probable explanation seems to be that the mother hen feels the danger as threatening herself as well as her chickens, or rather herself in her chickens, which form a part of herself. Under the influence of this strong affect she must utter her emotion. But here again her cry is instinctively understood by the young as announcing a danger, and has the same effect as if it were uttered with the intention to warn.

But there are also cases in which no strong emotion seems present in the crying animal, while yet the sounds uttered are understood as a communication by others.

This is for instance the case when the cock "calls" his hens on finding a piece of food. Must we admit here that the cock uttered his sound with the clear intention of calling his hens to the food? Many observers will do so, and compare the act of the cock with that of a good father who calls his children to show them a raspberry bush in the wood. Yet here too an explanation on a simpler basis seems possible. It is possible that the cock, as the leader of the herd, on finding the food only expresses his feeling of joy or satisfaction with this supply for the wants of his herd, which sound, however, on the side of the hens may secondarily have obtained the significance of an announcement of food. And when a mother cat, on coming back with a killed mouse to the place where she left her young and not finding it there, mews till her kitten comes to her, popular opinion will be inclined to admit that this mewling was done with the intention of calling her young. Yet it is possible that only a feeling of discomfort or deception at not finding her young at this moment impelled the mother cat to express her feeling of displeasure. That the mewling attracts the young is only a secondary effect, not the intention of the crying animal.

Perhaps it will seem to the reader that this explanation is rather far-fetched, and that it would be more plausible simply to admit that the cat calls her young as a human mother would have called her child in a similar situation. But we must not forget that in animal psychology the rule is still in force, originally formulated by Lloyd Morgan, which says that we may not explain an act of an animal as the outcome of some higher mental process as long as it is possible to explain it by a process that takes up a lower position in the scale of mental development. Especially on this subject, where laymen and even scientists are so often ready to overestimate the faculties of the animal mind, it seems wise to stick to this prudent rule.

As for the way in which such understanding of each other's expression of emotions may be acquired, we may admit that it is generally innate, in other words, the reactions to expressions of emotion by their fellows belong to the so-called instinctive reactions of animals. Against the assertion that such understanding would only be acquired by the experience of life we may submit that such reactions are to be found soon after birth, or even before birth, as is the case with the chicken in the egg which stops its early peeping when the mother hen gives the danger cry.

But this understanding of each other's expressions of emotion is not limited to the species. Animals of different species may in case of danger be warned by one another's danger cry. This is especially the case with animals often living together, as birds in a wood, or ostriches and buffaloes on the African plains, or chamois and marmots in the Alps. When the marmot expresses its fright in its particular whistle, the chamois grazing in its neighbourhood flees too. Such a mutual understanding may even take place between mammals and birds. The oxpecker *Buphaga* is a well-known companion to several big animals on the African plains, and not only rids them of parasites but also renders them the service of "warning" them of danger by a conspicuous showing of fright. Other examples of this kind of "symbiosis" might be quoted.

Now it seems questionable whether such understanding of animals of a different



species is innate. It seems more probable that such a "knowledge" of each other is gradually acquired by experience during life, in which imitation of the actions of parents by the young may play a rôle. A fact that speaks in favour of this is that when animals of different species that do not live together in the natural state are brought together (as is often the case in zoological gardens) at first there often arise difficulties between them, since the mutual experience of each other's sounds or gestures is wanting. The mandrill for instance when expressing a friendly attitude to another animal draws up the corners of its mouth so that the canines become visible. The baboon on the contrary shows its canines only in case of attack, and expresses an accommodating spirit by a quick smacking of the lips. So when both are brought together there is often a misunderstanding of each other's disposition, leading to the beginning of a fight. Furness<sup>(7)</sup> reports, on the contrary, that the chimpanzee understands the danger cry of the orang-utan, although it does not make this sound itself.

So although the sounds of animals are not uttered with the intention of communicating or expressing something to other beings, their sounds may be understood, either instinctively or by experience, by other animals. There may be members of their own species, or also animals of other species, and thus *secondarily* and *unintentionally* the sounds may serve as a means of communication.

Before leaving this subject we must face the question: Can the animal, in this same way either by experience or by training, learn to understand the language of man?

This case is somewhat different from that in which the mutual understanding of two animals is concerned. We found already as an essential difference between animal and human language that the words of human language have a conventional meaning, changing from one language area to another, and that they do not express feelings or emotions, but indicate objects or concepts. It would certainly mean a higher degree of intelligence if an animal could show itself able rightly to interpret human words than when it only shows an understanding of the emotional expressions of other animals. The question whether animals understand the meaning of our spoken words is not so easily answered as it would seem at first. Reactions of animals to human words will generally be determined rather by the intonation with which these words are spoken than by the meaning of the words themselves. Schmid<sup>(26)</sup> once proposed to his dog with a gay intonation and stimulating gestures to take him to the lethal chamber for dogs. At this proposition the dog jumped up and barked and showed all signs of joy. Schmid then told him in a sad voice that he had changed his mind and would let him live, at which announcement the dog showed signs of bitter disappointment and sadness. Although it is not always so plain as in this case, yet as a rule the reactions of our domestic animals to our words are rather influenced by our intonation than by the real sense of the words.

Yet *a priori* it is not at all impossible that an animal might learn to understand special human words, that is to form special associations with the acoustic sensations of human words. This is at least the case with domestic animals. It is generally admitted that higher animals (dogs, cats, horses) may be taught to respond to the

names we give them, *i.e.* may learn to distinguish between different sounds of human language and to react appropriately to one of these.

That the understanding of human words may cause rather serious difficulty to an animal like the cat was proved by experiments of Thorndike<sup>(24)</sup>. He trained a cat to climb up the walls of its cage for food, when he said: "I must feed those cats." But then the cat climbed just as well when the experimenter said: "My name is Thorndike." There was therefore no understanding of words, but only a general reaction to sounds spoken by the experimenter. Thorndike then tried to train the cat to react differently to the words "I must feed those cats" and "I will not feed them." On the latter no climbing was to follow. The words in these experiments were uttered as naturally as possible, so that there was not only a difference in the sounds, but also in the rhythm and the emphasis of the phrase as a whole. Yet it took about three hundred and eighty trials before the cat could distinguish between both phrases and did not climb up when the second phrase was pronounced. This proves that at least with cats a real understanding of and distinguishing between human words is possible, but rather difficult.

Now it cannot be denied that in Thorndike's experiments the sounds the cat had to react to were of a rather complex nature. Simpler experiments were performed by Shepherd. Shepherd<sup>(23)</sup> trained two cats to climb up the wall of their cage for food when their name was pronounced, while on the words "no feed," irregularly alternated with the pronunciation of the name no reaction was to follow. A young cat learned this after about two hundred and fifty experiments, an older one wanted about double the number of experiments before the association was formed. Shepherd made similar experiments with four raccoons<sup>(22)</sup>; in eighteen days, after 270-500 experiments respectively, these animals learned to react on the pronunciation of their name by climbing up for food, while on the pronunciation of other words reactions failed. That animals may thus learn to react on their name being spoken has at least been proved without a doubt.

A better understanding of, and sharper distinction between human words than is found in cats and raccoons seems to be found in dogs. When Edinger<sup>(4)</sup>, putting his hands in his pockets and looking away, pronounced the word "auto" or "tram," when walking with his dog, the animal was able to pick out the desired vehicle when both were in sight. Schiche<sup>(18)</sup> examined trained police dogs under severe precautions (the leader had to pronounce the order with his back turned to the dog, and his hands in his pockets, while the observer stood aside at a distance). Then dogs that were trained to sit down at the word "setz" and lie down at the word "Platz" did not react on the words like "seck," "plack," "retz" and the like. The dogs therefore were able to form associations with special words, and showed a fine distinction between rather similar ones.

As an interesting investigation of the question as to how far dogs understand human words, made with all possible precautions and on an animal that was reputed to know a great number of words of human language, we may mention a recent study of Warden and Warner<sup>(25)</sup> with the dog "Fellow." The owner of this dog asserted that it "knew" 400 words. Although in the critical examination the contention

could not be borne out, yet it appeared that the dog obeyed a great number of commands of his master, even when the latter was invisible to the dog, so that all unintended signs were excluded. It was clear therefore that by careful training a dog may learn to form associations with a great number of human words and is able to carry out the movements indicated by these words in the language of the speaker.

It is also a fact that a certain understanding of the meaning of human words may be acquired by some birds, especially by parrots. Below we shall return to the question of the pronunciation of human words by these birds; here I would only point to the fact that there are numerous indubitable instances of parrots associating the right word with the right situation, for instance pronouncing the word "come in" when there is a knock at the door. This proves that when these words were fixed in their memory the right human word was associated with the sound of somebody knocking at the door. For the present these cases do not prove more than the forming of associations between human words and other perceptions. For the assumption of a real understanding of the meaning of the words, going farther than the formation of simple associations between sense perceptions, there seems for the present no sufficient ground.

As the last characteristic of human language we have found that the words used in it are joined together to form continually new combinations, phrases with different contents, and that even, if necessary, new words may be formed to denote new objects. Human language, in other words, has a creative element, creative in the double sense of possessing the faculty to create new phrases out of words already extant, and in addition of creating new elements in the form of new words, by derivation, composition, etc. Animal language has neither of these faculties. That no phrases are formed by the animal is clear, since we saw already that the elements necessary for them, the words, are wanting. But even the cries of animals are stereotyped. They do not change when the animal comes into other conditions. We saw already that Boutan's gibbon used the gibbon-language just like its fellows. The language of the animal is stereotyped just as its instinctive actions are. And that animal language, even in such a relatively high animal as the gibbon, lacks all developmental faculty was shown by Boutan when he brought his animal into a world new to it: it was taught to eat with a spoon, drink out of a glass, sleep in a bed, etc. Yet no new sounds, related to anything in this new world, were formed by the animal and it clearly showed its incapacity of developing its vocal stock.

Perhaps somebody will raise the objection to this that birds' song shows some capacity for development. Young singing birds (nightingales, chaffinches) have to learn to sing their song and do this partly in imitation of older individuals, in consequence of which there may arise differences in the songs of birds of different areas and the song of the singing birds of one region may be of higher quality than that of another district. But the song of birds is not so much a direct expression of emotion as a playful activity, though it is true that the songs are generally based on some emotional experience. Birds' song cannot therefore be cited as a proof that animal language is capable of development.

So far we have only considered the sound production of animals. It would, however, be wrong to forget that they have still another kind of language, viz. attitudes of the body and gestures. The dog expresses its fear when, making itself as small as possible, it creeps on the floor with looks that seem to ask for mercy, and it expresses suspicion when it approaches a strange dog, walking upright with head slightly raised and tail erect, eyes and ears directed to the approaching animal. The horse expresses fright when, with ears on the neck, it turns back the pupils of the eyes, so that the white part of the eyes is visible, and it shows impatience when, while waiting, it paws the ground. And among invertebrates an animal such as the octopus expresses a number of emotions like fright or anger or desire for food by rapid changes in the coloration of the body.

Curious instances of this expression of emotion by bodily movements are to be found in insects. Some years ago von Frisch (5, 6), studying the life of bees, discovered that a bee when coming home after finding a rich store of food dances round in the hive and so stimulates other bees to follow her and go out in search of the food. Her dancing secondarily becomes a communication, just as was the case with the sound of the frightened animal in the herd. Another curious way of "communication" is found in the so-called antennal language of ants and other social insects. By tapping the head or antennae of a fellow ant in different ways an ant may express different emotions evoked in it by some event that has happened just before. Wasmann (26) composed a "vocabulary" of this antennal language and described a number of things that might be communicated in this way. Thus by tapping the head of a fellow ant with the antennae the attention of the congener may be drawn to special objects or activities, so that an imitation may be aroused that makes possible some common action in the colony, as for instance the changing of the nest or a predatory expedition. By tapping an ant expresses its hunger and so may induce a fellow ant to feed; the tapping may be an expression of fright at the perception of some danger and may therefore cause a common action against it, be it flight or attack. Thus the movements of the antennae may have the same result with ants as the vocal expressions have with higher animals.

Bodily attitudes and movements further play a great rôle in expressing sexual emotions. It is well known that a great number of animals when sexually stimulated give expression to this by special attitudes and movements, which may sometimes develop into complicated dances. The cause of all this display in lower and higher animals is the need to express special emotions and when they are understood as such by the females they form a kind of language, just as sounds do.

The question that interests us now is: May not this gesture language have some qualities by which it reaches a higher level than sound-language and does it approach more nearly to human language, as the prototype of real language? That it cannot be articulate, cannot have words, is clear at once. But has it the quality of being intentional? Have the movements or attitudes learnt during life got conventional meanings? Do they indicate special objects or do they only express emotion? Do animals invent new ways of bodily expression from time to time? The answer to these questions is not difficult to find. Gesture language takes up no

higher position than sound language in the animal world. Movements and attitudes are assumed instinctively; that is, they are innate and typical of the species and are more or less stereotyped for all animals of the species. They are not premeditated, nor assumed with special intention in the presence of other animals. They only express feelings, just as the sounds of animals do. This is especially clear in the case of the bee, where the dancing fails when only a poor store of food is discovered and the emotion caused by it is only feeble. This is even the case in sexual display, which is often carried out when no female is in the neighbourhood, or before an animal of a different species or a plant or even a lifeless object, as for instance the food-cup in the case of birds kept in confinement<sup>(1)</sup>. There is no reason to estimate the gesture language in animals as a higher accomplishment than the sound-language.

So far we have only compared animal language with human language in its highest form, as an act of intentional communication of ideas and feelings by means of articulate sounds. But we must not forget that in man too there exists a lower type of expression more corresponding to the language of animals, as we have learnt to understand it now. Man too has his "pseudo-language," vocal as well as non-vocal. Under the influence of some strong emotions man may exclaim "oh" or "ah" in the same way as the animal cries out his fear and anger. And as Boutan rightly remarks, this pseudo-language in man also corresponds with the language of animals in this respect that these sounds are immediately understood by other men, and also that they may express more than one emotion, so that it is chiefly by their intonation that their meaning is understood. The same holds good for man's attitudes of fear or rage, for his weeping or laughing, all expressions of different feelings, uttered (most of them, at least) without the intention of communicating anything, but secondarily becoming a means of communication since they are understood by other men. This pseudo-language of man is quite comparable to the language of animals and, just as is the case in the animal world, appeals rather to the sentiments of the bystanders than to their intelligence.

So far the result of our enquiry has been that animal language in all its essential features is inferior to that of man. It is but rarely vocal, never or hardly ever articulate, and has no words in the sense of sounds bearing a conventional meaning. The sounds do not indicate anything, but they express feelings and emotions. They are uttered unintentionally, showing no capacity for development. And the language of attitudes and movements does not seem to reach a higher level. But now the question arises: Can the natural language of animals reach a higher level under the influence of man and in intercourse with him? May it even by experience or training become a real language of the same nature as that of man? Here again we must consider the question from the technical, sociological and psychological sides.

Can an animal be trained to produce articulate sounds? Since in its natural state, as we saw before, no animal produces articulate sounds, this question reduces itself to this: Are animals, or some of them able to imitate man and to pronounce some of his words, quite apart from the question as to whether they attach the right meaning or any meaning at all to them?

This question has been answered in the affirmative for the dog. As early as 1715 Leibniz described a dog that could talk with human words. Afterwards other dogs followed, and more recently a certain fame was acquired by a dog called Don which could pronounce its own name and was said to be able to answer certain questions with words such as "Hunger," "Kuchen" (cake), "Ruhe" (rest), "haben" (to have) and Haberland (a proper name). It was said to have learnt these words by spontaneous imitation. However, Pfungst<sup>(17)</sup>, who carefully studied this dog, came to a lower estimation of its faculties. First of all it appeared that the dog did not at all understand the meaning of the words, and often answered to questions with the wrong word. But also, a point that is of greater interest here, the sounds produced by the dog proved on closer examination to have only a faint resemblance to the human words they were supposed to represent. By analysis of phonographic records of the sounds of the dog it appeared for instance that out of thirty-four sounds which were interpreted as "Don" (the name of the dog) more than 50 per cent. were two- or three-syllabic. The so-called words were simply the natural sounds of the dog, inaccurately observed and judiciously interpreted under the suggestion of the owner. In this connection we are struck by the fact that it is always the same words that are pronounced by such "talking-dogs." German dogs always say: "haben" and "Kuchen," and what in this dog was believed to be the family name "Haberland," in a dog described by Romanes was interpreted as "Grandmama." There are no more human words in the sounds of this dog than there are in the natural sounds of some birds, as for instance the song-thrush, in which some bird lovers like to hear human words.

But what about monkeys, so nearly related to man? Are they able to learn to pronounce human words? Experiments of this kind were made twice, viz. once by Yerkes<sup>(27)</sup> with a chimpanzee, and once by Furness<sup>(7)</sup> with chimpanzees and oranges. The results of Yerkes were completely negative, although he tried four different methods during a period of eight months. Different devices were constructed, by which a piece of banana fell down through a hole on a table, or was uncovered by the raising of a box, while the experimenter pronounced words like "ba-ba" or "co-co." But although the ape could take the banana when it appeared, he never made any sound that was an attempt to imitate the sound of the experimenter. Somewhat better were the results of Furness, who tried to teach a young chimpanzee and an orang-utan to pronounce certain words. He tried to do this in the same way as is done with deaf and dumb children, viz. by opening and closing the lips of the animals before a mirror, while he made the same movements with his own lips. An attempt to teach the chimpanzee to say the word "Mama" failed however completely. Better results were obtained with a young orang, which was a better pupil than the chimpanzee because of its greater patience and lesser irritability. This animal learned to say "Papa," and pronounced it suddenly after six months of training. Gradually the animal learned to associate this word with the experimenter, so that it was regarded more or less as his proper name. In the same way Furness succeeded in teaching the animal how to pronounce the word "cup," while at the same time a drinking cup was shown. Furness describes how in this way the word



"cup" became associated with the cup itself, so that the animal came to call the word "cup" when it was thirsty. This is certainly a very remarkable result, to which I shall return below. It was a great pity that the animal died before more extensive results had been obtained.

It is certainly a curious fact that an accurate reproduction of human words is found not so much in animals nearly related to man like the higher monkeys, but rather in lower animals, viz. the birds. The reason must be that while monkeys show a tendency to imitate movements perceived visually, they have no tendency to imitate sounds perceived acoustically. Therefore we must look for a reproduction of human sounds among animals which, although less nearly related to man and certainly much less mentally gifted than apes, show this tendency of imitating acoustic perceptions.

A number of birds are known to be able to pronounce human words in a fairly accurate way. Instances are known of canaries and bullfinches (*Pyrrhula pyrrhula*) that were able to pronounce whole phrases. Among the starlings the beo (*Eulabes*) and among the Corvidae the raven seem to merit the first prize.

But the highest prize is due to the family of parrots, and among them especially to the grey parrot (*Psittacus erythacus*). This grey parrot has a remarkable faculty for reproducing and retaining human words. There was one individual, that lived in Europe from 1827-1854 and was always with masters who tried to develop its speaking faculties, that probably attained the greatest proficiency in this respect. After its death it was described by Brehm (3), who mentions a great number of words and phrases this good disciple learned from its ardent teachers. Some years ago Lashley (13) described an Amazon parrot (*Chrysotus* species) that possessed a vocabulary of fifty to one hundred distinctly articulated words. Von Lucanus (15) praises the skill of the grass parakeet (*Melopsittacus undulatus*) in pronouncing phrases of three and four words after a few days' teaching. One of them in three days learnt to say: "Ich kann schön sprechen," and learned in a short time to count up to ten, etc. As far as concerns the technical side of the question the parrots certainly reach a higher level of language than any other animals, not even excepting the isolated case of Furness's orang. Below we shall see that they also reach a higher level from another point of view.

Now that we know that at least some animals are able to learn to pronounce articulate human sounds, we must ask ourselves the following question: is it possible that these sounds become real words to them, i.e. obtain a conventional meaning and indicate in this way some special object or situation? We must admit this for Furness's orang, for which the word "cup" really had a special acquired meaning and indicated the object that contained its drink. We must admit this too for parrots, which often give evidence of joining a special meaning to the words they have learned to pronounce. When a parrot says "good morning" only when it sees some person for the first time on a certain day, and says "good evening" only at the end of the day, as has often been described, then it is clear that the bird associates a special situation with these words, even when it is doubtful if it understands the meaning of the words "good," "morning" and "evening" separately. Von Lucanus

described a parrot that had formed the habit of saying "so" when some act was finished, and "na" when it was in the expectation of something being done. And it said these words not only when some act was finished that had some relation to the bird itself, as for instance the closing of its cage, but also when a lamp was put on the table, etc. The animal, therefore, by these little words indicated special situations, and, what was especially interesting in this case, it came to extend the meaning of the word from a special to more general situations of ending or commencing acts. Anyhow this shows that parrots may acquire real words: articulate sounds with conventional meanings, indicating special objects or situations.

But now another question arises. We have seen that a difference between animal and human language is that while the latter intends to communicate something this is not the case with the former. The animal only expresses its emotions, has no desire to communicate anything. May it be that in the intercourse with man this changes, and that a desire to communicate ideas appears in the language of the animal?

As there is no reason here to separate sound-language from gesture-language, we may treat them together. As a matter of fact, in the intercourse with man some of our domestic animals learn by experience to express special wishes and desires by special attitudes or sounds. The best known example of this is the "begging" of dogs. The special attitude of sitting down on the hind quarters and holding the fore legs up horizontally is not a natural expression of any feeling of hunger in the dog. By accident or by training however the dog has learnt that this attitude is rewarded by a titbit. The attitude is wholly conventional for the dog; no dog will ever understand why it is exactly this attitude that is rewarded by its master. But when after some time the attitude has been rewarded often enough, the dog not only associates attitude and reward, but even provokes the reward by assuming the attitude. Then a higher level of gesture language is reached: the conventional attitude is assumed to express his wish for the titbits. This kind of gesture language is more nearly related to the language of man than the simple expression of fear or joy.

Many other instances of the same type of language might be quoted. The cat that stands mewling before a door it wishes to be opened, the first time it did it certainly merely expressed some feeling of displeasure. After it had experienced that a certain number of times, on its mewling, a human being came and opened the door, it learnt to mew with the intention of invoking this help of its master. Dogs jump before a door they wish to be opened by man, and Yerkes trained a young chimpanzee to "speak for food," as dogs do. Some of Thorndike's cats were trained to lick or scratch themselves in order to have the door of their cage opened. Their licking and scratching thus acquired the meaning of a request to be set free. The conventional character of all these signs goes without saying. Animals in zoological gardens often show such expression of their wish for rewards: bears dance for the cakes of the visitors, and in the gardens at Amsterdam there is a female monkey that has formed the habit of quickly turning round and round and then stretching out her hand in order to beg for food.

This expression of wishes by domestic animals is not limited to attitudes or movements alone. I have known a dog that had formed the habit of whining in a special way when it wanted to drink. This whining was only performed for that purpose. It was gradually developed and was certainly not innate. Edinger tells something similar about his dog.

But now the question arises: Is such an intentional utterance of special sounds, is such an intentional assumption of special attitudes limited to intercourse between animal and man? Does it never happen, that in its intercourse with its own fellows the animal experiences the results of its sounds or movements and learns to use them as a means to communicate wishes?

It seems difficult to give a definite answer to this question. Theoretically it may be possible that for instance in the intercourse between mother and young ones one party experiences that some unintentionally uttered cry always has the same effect on the other party and so after some time it may come to utter the sound with the intention of producing the effect, just as the begging dog did. Some authors are inclined to admit this, perhaps rightly. Yet we must take care not to confound with this the anticipatory movements that are sometimes carried out by animals strongly desiring to obtain a certain result. When for instance a monkey in a zoological garden stretches out its hand to receive a piece of fruit from a visitor, this is not to be understood as a conventional movement, as was the case with the dog sitting up and begging, but as an endeavour to catch the food seen in the hands of the visitor. Of course this movement may be stereotyped afterwards and then come to be more or less like the attitude of a beggar holding up his hands for alms, and so form a transition to the conventional movements described above. But it will be clear that at least in its origin it had a more natural significance.

The same now may happen between mother and young. When the mother bird comes back to the nest, it has often been described how the young birds "beg for food." Mostly this only consists in an opening of their bills and crying while sometimes the young birds go farther and seize hold of their mother's bill. It will be clear that these acts are not comparable to that of the dog sitting up to beg for food. There is no intention to express a feeling of hunger to the mother bird, but by anticipatory attitudes or acts the young bird tries to get from its mother all that it possibly can. There is no more wish to communicate anything here than there is in the act of the monkey which for the first time of its life stretches out its hand to catch a fruit held up before it by a visitor.

I have quoted these examples to show that we must walk with circumspection here and not take for communication what is only an endeavour to obtain a result desired. The question whether animals in their natural state utter any sounds or assume any attitudes with the intention of communicating something to other animals remains unsolved. As far as I know there never was a small dog that "sat up" before a bigger dog to obtain a bone that the bigger one possessed, and this fact certainly argues against the supposition. These conventional attitudes seem to be restricted to the intercourse of the animal with man alone.

We have seen that some animals at least are able to pronounce articulate sounds

and to attach some conventional meaning to them; in other words, that some animals use real words. We have also seen that other animals may be induced to express their wishes intentionally by assuming certain attitudes or uttering certain sounds. Does it happen that some of the first class reach the level of the second? In other words, are there any animals that can use human words to express intentionally something they desire to communicate?

As far as I know only two examples of this faculty are known. The first is the somewhat dubious case of the orang of Furness, mentioned above, which came to pronounce the word "cup" when it was thirsty and wanted its cup to drink. If this case is true, we have here an example of language of a high class in animals, although perhaps only rather an elementary one: the animal wishes to communicate something and does this by means of an articulate word with a conventional meaning. But Furness himself considers his result unsatisfactory, as the animal died before more was attained. And as this is the only orang in which such a feat has been reported, we shall have to wait for more examples before deciding that the orang is able to speak such a high type of language.

With parrots the case is different. We saw how well certain species of parrots can be taught to imitate human words. Yet the reproduction of words is generally no more than an act of pure playfulness. Usually the animal does not associate its sounds with special objects or situations, but blurts out the words and phrases it has learned without any deeper understanding. In such cases there is no wish to communicate, no using of words with a special intention. Only the correct pronunciation of articulate sounds, the technical side of real language, is present.

But there are also cases in which the language of parrots reaches a higher level, not only in that the words seem to be understood by the animal, as we have seen above, but also in that they are uttered intentionally. Many stories and anecdotes are quoted of parrots showing this ability to pronounce the right words at the right moment in order to obtain an effect desired. The greater number of these stories, however, are of somewhat doubtful origin, communicated as they are by owners who wish to tell a good story about the intelligence of their pets, and it is often difficult to separate the wheat from the tares here. Many of them too are wrongly interpreted. When for instance a parrot cries "come-in," when somebody is knocking at the door, there is no reason to suppose that the bird really desires to express a wish to see the person enter the room. The knocking at the door has always been followed by a "come-in" from its master; in the absence of its master the bird now completes the sequence of sounds. There is no reason to seek for more in its words.

As I am restricting myself to observations of scientific observers, I can only quote two instances where parrots seemed intentionally to communicate something to other beings by means of human words. The first example is quoted from Hachet-Souplet (10). He taught a parrot the word "armoire" while pointing to a little cupboard against the wall where the birds' food was put away, "échelle" for a ladder, and "monter" for the act of climbing this ladder. The cupboard was hung high against the wall, so that it was necessary to mount the ladder to reach it. One morning the parrot was not fed, and then began to cry "moire, moire" (armoire),

probably excited by the vision of the cupboard with the food. As the bird had to wait longer for its food, it became furious and cried suddenly "*chelle-monter-armoire*." This seems at first sight a very curious performance on the side of the animal. First because it had not been taught the phrase, but had composed it itself. It seems to prove high intelligence in the animal, as the word "*armoire*," the principal purpose of the animal, was preceded by the word "*échelle*" as indicating the means to reach it. Perhaps we may doubt whether a parrot is really able to understand the relation of these things, to see that the ladder was a necessary means for its master to reach the cupboard. But a more serious criticism against the interpretation of Hachet-Souplet, who believed that the animal used the words with full understanding, is that he had taught only three words to the animal, so that it is possible that the latter at this moment of emotion blurted out its whole repertory. If the parrot had disposed of a richer repertory and yet at this moment had only used these three words, then this case would have been much more convincing, and certainly would have proved a high level of linguistic capacity.

Better instances were given by von Lucanus<sup>(14,15)</sup>. A parrot that was taught to say "*bitte*" when cherries were offered to it, afterwards pronounced this word at the sight of all kinds of food, and in the end even used the word to express the will to get some food or special object. Von Lucanus, who is an accurate observer and a very cautious interpreter, does not believe that a will to communicate anything is present here, because the animal used the word also when nobody was in the room. I believe that this only proves that the parrot did not wish to communicate his wishes to special persons, just as the man who falls into the water may cry for help, even when nobody is in sight. Another parrot, which had learned to say "*adieu*" when anybody was going away, afterwards began to call "*adieu*" when somebody was in the room whose presence was disagreeable to the animal (it is a well-known fact that parrots often have strong dislikes against special persons). This was done before that person showed any sign of departing. There seems to be no question but that here we have a high type of animal language: a word with special conventional meaning, used by an animal with the intention to communicate something to somebody!

It cannot be denied that our examples are only few in number, and that our knowledge of this highest form of animal language is rather meagre. It would of course be possible to quote other examples from the literature, but, as stated before, their trustworthiness is not always certain; so it is better to err on the safe side by being rather too cautious and critical than the reverse. Anyhow it is to be hoped that in the future the study of a bird so easily kept as the parrot will furnish more proofs of the occurrence of this type of language in animals.

Our last question then must be: Does animal language ever reach the level of real language, as we defined it in the beginning of this article? Does an animal ever really speak as man does? We shall not be too bold if we assert that not only is no example of this known, in spite of all that is told in legend and fairy tale, but further that no animal will ever get as far as that. Real creative language, the faculty of combining words into phrases, is beyond the faculties of the animal mind. Even in

contact with man, animal language in its highest form remains the language of an animal. It is only the human mind that possesses the full faculty of combining words into phrases, and phrases into conversations, of constantly creating new means to express and communicate its feelings and thoughts. It seems that an unbridgeable gulf here separates man from other animals.

In this article we have only drawn comparisons between the language of the animal and of adult man. It would be tempting to go one step further, and compare the language of animals also with that of growing man, the child. So Hobhouse<sup>(12)</sup> put the language of the child of about one year on a par with that of animals, such as we have got to know it in the preceding pages: at this age it responds to special perceived words with an act, just as the dog does, and to certain perceptions with a word, just like the parrot. The language of the child does not seem to reach the level of real language before the end of the second year; only then two or more real words are put together to communicate something. But I believe it is better not to follow this line. The aim of this article has been chiefly to describe the characteristics and development of animal language, not that of man. I hope it has become clear that this problem of the language of animals is worthy of the attention I have asked for it, and merits further study on the part of biologists and animal psychologists.

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# DIE INNERE SEKRETION BEI WIRBELLOSEN TIEREN

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(Received April 2, 1929.)

(With Nineteen Text-figures.)

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## EINLEITUNG.

SOVIEL über die Morphologie, Physiologie und Korrelation der endokrinen Drüsen bei Wirbeltieren bereits gearbeitet wurde, so wenig wissen wir über die Inkretion bei wirbellosen Tieren. Nur in ganz wenigen Fällen sind inkretorische Vorgänge mit Sicherheit nachgewiesen. Vielfach bestehen nur Vermutungen über die inkretorische Bedeutung dieser oder jener Drüse. Noch vor nicht all zu langer Zeit gab es Forscher, die ein Vorkommen echter Inkretion bei Wirbellosen überhaupt nicht in Betracht zogen. Und wenn auch der bis heute vorliegende Wissensstoff auf unserem Gebiete noch garnicht ausgedehnt ist, so lohnt es doch, ihn bereits jetzt ein erstes Mal mit möglichster Vollständigkeit zusammen zu tragen, und wenn dies auch nur zu dem Zwecke geschähe, weitere Forscherkreise auf dieses noch unbebaute Arbeitsfeld hinzuweisen.

## DARSTELLUNG DER EINZELGEBIETE.

## DIE GESCHLECHTSHORMONE.

Ähnlich wie bei den Wirbeltieren waren es auch bei den Wirbellosen gewisse Geschlechtscharaktere, die zuerst zu einer Annahme innersekretorischer Vorgänge führten. Die Fragestellung war dabei im allgemeinen die: welche Korrelationen bestehen zwischen den Keimdrüsen und den sekundären Geschlechtsmerkmalen? Zur Beantwortung dieser Frage diente die Beobachtung der Änderungen sekundärer Geschlechtsmerkmale, die nach Kastration auftreten. Dabei kann die Kastration durch parasitische Einflüsse oder durch das Experiment bedingt sein. Zusammenfassungen dieses Teilgebietes finden sich bei Biedl (1916) und vor allem bei Harms (1914 u. 1926). Dem zeitlichen Gang der Forschung folgend besprechen wir zuerst die *Wirkungen parasitärer Kastration*.

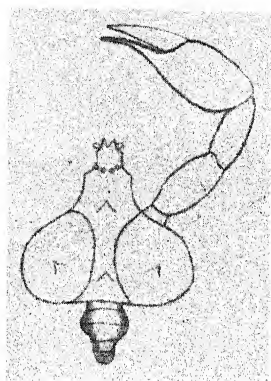
## PARASITÄRE KASTRATION DER KRABBen DURCH RHIZOCEPHALEN.

Bekanntlich haben die zu den Cirripediern gehörigen Rhizocephalen (vor allem *Sacculina* und *Peltogaster*) im erwachsenen Zustand eine rein parasitäre Lebensweise. Ihre Wirtstiere sind dekapode Krebse wie z. B. *Carcinus*, *Inachus*, *Pagurus* u.a. Das Wirtstier wird schon in frühester Jugend von dem Schmarotzer befallen. In der Folge durchsetzen wurzelartige Ausläufer des Parasiten den Wirtskörper in allen Richtungen. Die Rhizocephaleninfektion bewirkt im Wirtskörper eine vollständige oder teilweise Zerstörung der Geschlechtsorgane und ihrer Ausführwege.

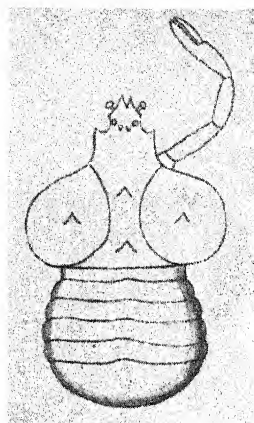
Im Jahre 1885 beobachtete Malm, dass der sonst unverkennbare Geschlechtsdimorphismus männlicher und weiblicher Krabben mehr oder minder schwindet, wenn die Tiere von *Sacculina* infiziert sind. Nahezu gleichzeitig begann auch Giard (1886) seine Untersuchungen über den Einfluss der parasitären Kastration auf die sekundären Geschlechtsmerkmale der Krabben. Ungefähr 10 Veröffentlichungen (1886–1904) hat Giard diesem Gegenstand gewidmet. Die Ergebnisse seiner Arbeit wurden vertieft durch die Forschungen von G. Smith (1906, 1910), Potts (1906), Nilsson-Cantell (1927), u.a.

Die Veränderungen der äusseren Geschlechtsmerkmale, die an parasitär kastrierten Krabben zu beobachten sind, machen wir uns am besten an Hand von Tabellen klar (vgl. auch Abbildung 1 u. 2). Tabelle 1 soll zunächst zeigen, dass bei den normalen nichtinfizierten Krebsen deutliche Geschlechtsunterschiede im Bau des Abdomens, in Zahl und Form der Abdominalanhänge und in der Grösse der Scheren vorhanden sind. Ferner soll zur Darstellung gebracht werden, dass von *Sacculina* befallene Männchen sich deutlich dem weiblichen Typus annähern, infizierte Weibchen hingegen nur geringfügige Veränderungen ihrer äusseren Geschlechtsmerkmale erkennen lassen.

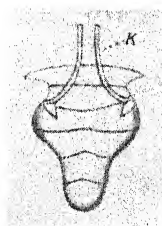
Tabelle 1 möge durch einige Zahlenbeispiele ergänzt werden. Es sind Durchschnittswerte in mm, die sich aus den ausführlichen Tabellen von Smith (1906) entnehmen lassen.



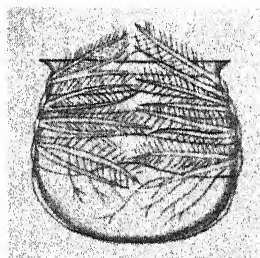
a



c



b



d

Abb. 1. *Inachus mauretanicus*. Normale, nicht infizierte Tiere. (a) Erwachsenes Männchen (breite Schere, kleines Abdomen). (b) Unterseite des Abdomens von einem erwachsenen Männchen. (c) Erwachsenes Weibchen (schmale Schere, dickes Abdomen). (d) Unterseite des Abdomens von einem erwachsenen Weibchen (Anhänge). K Kopulationsorgan. (Nach Smith.)

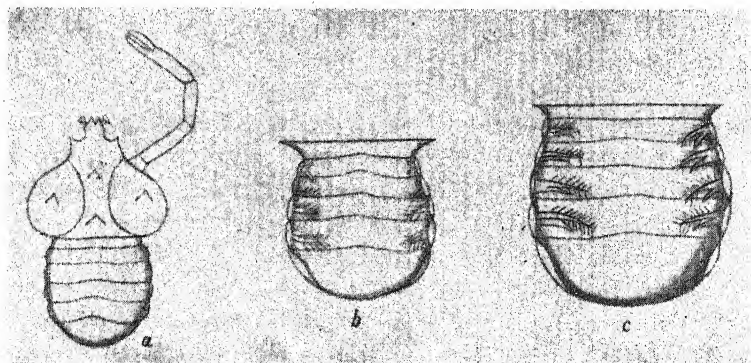


Abb. 2. *Inachus mauretanicus*. Infizierte Tiere. (a) Infiziertes Männchen, das vollständig weibliches Aussehen bekommen hat. (b) Abdomen (Unterseite) des gleichen Männchens. Weitgehende Verkümmern des Kopulationsorganes, Auftreten weiblicher Anhänge. (c) Abdomen (Unterseite) von einem infizierten Weibchen. Keine Änderung der Gestalt, lediglich Rückbildung der Anhänge. (Nach Smith.)

Tabelle 1. *Inachus mauretanicus*.

	Abdomen	Abdominalanhänge	Scheren
Männchen normal	Klein	2 Paar Anhänge (a) Kopulationsorgan (b) Reduz. Extremitäten- anhang	Lang u. dick
Männchen v. <i>Sacculina</i> infiziert	Vergrössert	2 Paar Spaltfüsse mit Haaren, Kopulationsstilet zu einem Knopf reduziert	Vershmälerte kleine Scheren
Weibchen normal	Breit und muldenförmig	4 Paar Spaltfüsse mit langen Haaren	Schmal u. klein
Weibchen v. <i>Sacculina</i> infiziert	Ähnlich wie normal	Spaltfüsse kleiner, Haare kürzer	Ähnlich wie normal

Tabelle 2. *Inachus scorio*.

	Länge des Abdomens	Carapaxlänge	Breite der Scheren
Männchen normal	6	12-13	3-4
Männchen infiziert	8-11	14	2-3
Weibchen normal	13-15	14-17	—
Weibchen infiziert	11-15	14	—

Bei etwa Dreiviertel aller infizierten Männchen lassen sich diese Angleichungen des männlichen an den weiblichen Habitus erkennen. Es ist selbstverständlich, dass lange nicht in allen Fällen die parasitäre Kastration so starke Veränderungen bewirkt, wie sie in Tabelle 1 angegeben sind. Alle möglichen Uebergangsformen vom nahezu unveränderten bis zum weitgehend verweiblichten Männchen sind gefunden worden. Diese Verschiedenheiten im Grade der Geschlechtsumstimmung hängen vor allem davon ab, in welchem Alter das Wirtstier vom Parasiten befallen wurde. Es ist klar, dass bei jungen Krabben, deren Geschlechtsmerkmale noch wenig entwickelt waren, die parasitäre Kastration viel stärkere Abänderungen im Gefolge hat, als bei älteren Tieren.

Die verschieden starke Ausprägung der äusserlich wahrnehmbaren Kastrationsfolgen hängt aber auch unmittelbar von dem Grade der Gonadenzerstörung ab. Treten z.B. beim infizierten Männchen weibliche Spaltfüsse auf, so ist zwar eine starke Schädigung der Hoden erfolgt, die Vasa deferentia sind aber noch erhalten. Ebenso lassen sich noch Spermatozoen nachweisen. Sind aber die Drüsen samt ihren Ausführungsgängen vollständig vernichtet, dann ist auch die Umstimmung der äusseren Geschlechtsmerkmale eine nahezu vollständige: die Männchen sind dann nur noch an dem knöpfchenartig verkümmerten Begattungsorgan zu erkennen.

Wissenswert ist fernerhin die folgende Tatsache: nach künstlichem oder natürlichem Abbruch der Infektion erfolgt eine Neubildung der Keimdrüsen (Smith). Dabei entstehen nun bei einem parasitär kastrierten Männchen nicht nur männliche, sondern auch weibliche Keimdrüsen. Es lassen sich nach erfolgter Regeneration

nicht nur reife Samenzellen, sondern auch durchaus normale Eier nachweisen. Die Krabben sind also Zwitter geworden. Harms weist darauf hin, dass bei Fröschen (nach Meyns) dieselben Erscheinungen zu beobachten sind: nach Kastration und folgender Transplantation reifen Hodengewebes gehen alle Keimzellen bis auf die Urkeimzellen zurück. In den sich neu bildenden Tubuli treten Samenzellen und Eier auf.

Eine wesentliche Erweiterung dieser Tatsachen brachten Beobachtungen von Courrier (1921): bei 66 infizierten *Carcinus*-Männchen fand er zwar 46 Tiere, die äusserlich ein weibliches Abdomen zeigten, aber nur bei 4 Krabben waren die Keimdrüsen völlig zerstört. Bei der Mehrzahl der Tiere waren nun bei völlig verweiblichten Abdomen die männlichen Keimdrüsen durchaus funktionsfähig, in den Vasa deferentia fanden sich Spermatophoren. Im Anschluss an die Untersuchungen von Pézard, Lipschütz, Ottow und Wagner über partielle Kastration kommt Courrier darum zu dem bedeutsamen Schluss, dass die *éléments séminaux* keinen Einfluss auf die äusseren Geschlechtsmerkmale haben. Ihre Ausbildung ist vielmehr bedingt durch die Tätigkeit eines inkretorischen Organs, das physiologisch und möglicherweise auch anatomisch von den Keimdrüsen unabhängig ist.

Die Bedeutsamkeit des Courrierschen Befundes wird uns sofort klar, wenn wir kurz die ziemlich verwickelten theoretischen Erörterungen betrachten, die an die Erscheinungen der parasitären Kastration angeknüpft wurden.

Smith zieht aus seinen Beobachtungen folgende Schlüsse: es sei zwar eine unmittelbare Abhängigkeit der sekundären Geschlechtsmerkmale von den Keimdrüsen anzunehmen. Die Wirksamkeit der Keimdrüsen sei aber nicht eine inkretorische, sondern beruhe auf einer "Geschlechtsbildungssubstanz" ("sexual formative substance"). Diese hypothetische Substanz wirke nun vermittels der in Blut und Leber vorhandenen Fette (Smith, Robson (1912)). *Sacculina* bewirkt nämlich ebenso wie die Eireife bei einem normalen Weibchen eine Erhöhung des Fettgehalts. Und eine Erhöhung des Fettgehalts übt—so folgert Smith—auf das Männchen einen verweiblichenden Einfluss aus. Diese Schlussfolgerung Smiths ist wohl nicht ohne weiteres bindend: die beim infizierten Männchen pathologisch auftretende Fettanreicherung hat möglicherweise eine ganz andere Ursache als die Fettanreicherung beim normalen Weibchen. Infolgedessen ist es auch nicht unbedingt notwendig, der Fettanreicherung bei normalem Weibchen und infiziertem Männchen die gleiche Folgeerscheinung—nämlich Ausbildung äusserer weiblicher Geschlechtsmerkmale—zuzuschreiben.

Wohl mit Recht weist Harms (1926, S. 311) darauf hin, dass "kein grosser Unterschied zwischen der Theorie der formativen Substanzen und der inneren Sekrete" bestehe.

Biedl (1916, II, S. 225) bemerkt im Anschluss an seine Darstellung der parasitären Kastration durch Rhizocephalen, dass es sich bei diesem Naturexperiment nicht nur um eine frühzeitige Zerstörung der Keimdrüsen, sondern um eine bei den Krabbenmännchen erfolgte Transplantation einer weiblichen Keimdrüse handle. "Indem die eingewanderten Parasiten nur Weibchen sind," sagt Biedl wörtlich, "welche im Wirt geschlechtsreif werden, entfaltet sich nunmehr der Einfluss der weiblichen Keimdrüse des Schmarotzers und wirkt auf die Entwicklung der sekun-



dären Geschlechtscharaktere des Wirts bestimmend." Harms (1926, S. 311–12) schliesst sich der Meinung Biedls voll und ganz an. Er führt zu ihrer Begründung noch die Tatsache ins Feld, dass Krabbenweibchen, die vor Eintritt der Geschlechtsreife von *Sacculina* befallen wurden, vorzeitig die Merkmale von ausgewachsenen Weibchen annehmen. Harms sagt: "Da die Gonade selbst aber durch den Parasiten zerstört ist, so kann nur das innere Sekret der weiblichen *Sacculina* diese Beschleunigung in der weiblichen Richtung bewirken."

Eine m.E. bedeutsame Klärung hat die ganze Frage durch die Ausführungen van Oordts (1927) erfahren. Er weist vor allem darauf hin, dass die Erklärungsweise von Biedl und Harms, die sich auf die "Transplantation einer heterosexuellen Keimdrüse" stützt, deshalb nicht haltbar ist, weil die Sacculinen, wie ja die meisten Cirripedier überhaupt, Zwitter sind. Die infizierende *Sacculina* ist also garnicht—wie Biedl meint—ein Weibchen, es kann also auch kein weiblich umstimmender Einfluss vom Parasiten ausgehen.

Nach Ablehnung der Smithschen Erklärungsweise zieht van Oordt einen Vergleich zwischen den Folgeerscheinungen der parasitären Kastration bei den Krabben und der Ovariectomie bei Haushühnern. Van Oordt bezieht sich da in der Hauptsache auf die neuen Untersuchungen von Domm (1927). Die Beweisführung van Oordts lässt sich am kürzesten in einer Tabelle darstellen. (Vgl. Tabelle 3.)

Tabelle 3. Vergleich der Kastrationsfolgen bei Haushuhnweibchen und *Inachus*-Männchen.

	Äuss. Merkmale	Wirkung des Kastrationsgrades	Regeneration	Neutraler Typus
Haushuhn nach Ovariectomie	Federkleid des Kapauns	Es gilt das Alles- oder Nichtsgesetz (Schwelle innerer Sekretion)	Aus dem rechten Ovar entwickelt sich ein Hoden	Dem männl. Geschlecht angenähert
<i>Inachus</i> männchen nach parasit. Kastration	Weiblicher Habitus	Der Grad der Umstimmung äusserer Merkmale ist bedingt durch den Grad der Kastration, bezw. der Menge der sezernierten Geschlechtshormone	Es regenerieren weibl. Geschlechtsorgane	Dem weibl. Geschlecht angenähert

Der Schlussstein in seiner Erklärung ist also der, dass beim Haushuhn die männliche, bei *Inachus* aber die weibliche Form dem "neutralen" Tier am nächsten steht. Infolgedessen nimmt bei Wegfall der Geschlechtsinkrete, die die äusseren Merkmale bestimmen, das Huhn männlichen, das *Inachus*männchen aber weiblichen Charakter an. Und aus dem gleichen Grunde sind am parasitär kastrierten *Inachus*weibchen keine wesentlichen Veränderungen zu beobachten.

Zusammenfassend können wir sagen, dass—abgesehen von Smith—alle Forscher die bei parasitärer Krabbenkastration auftretenden Folgeerscheinungen durch Veränderungen der inkretorischen Vorgänge erklären. Der Bildungsort der Inkrete ist nicht festgestellt. Nach Courrier steht er mit der Geschlechtsdrüse selbst nicht in physiologischem Zusammenhang. Es kann nicht deutlich genug darauf hingewiesen

werden, dass die Annahme von Geschlechtshormonen bei den Krabben—so gesichert sie auch erscheint—einstweilen nur auf Hypothesen und Analogieschlüssen beruht. Ein unmittelbarer Nachweis von Sexualhormonen der Krabben ist bis heute noch nicht erfolgt.

Nachdem an dem Beispiel der von Rhizocephalen befallenen Krabben (vor allem *Carcinus* und *Inachus*) die Hapterscheinungen der parasitären Kastration dargelegt sind, sollen nur noch einige weitere Fälle parasitärer Kastration besprochen werden.

#### WEITERE FÄLLE PARASITÄRER KASTRATION.

*Paguriden und andere Krebse.* Giard (1887), Guérin-Ganivet (1911) und schliesslich Nilsson-Cantell (1926) haben eingehend die von *Peltogaster paguri* und *sulcatus*

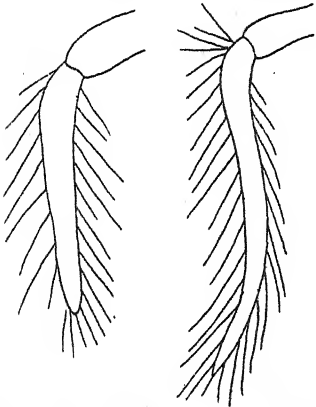


Abb. 3.

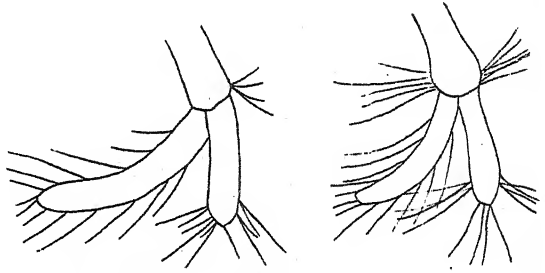


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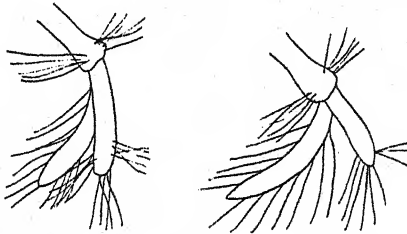


Abb. 5.

Abb. 3. *Anapagurus chiroacanthus*. Nicht infiziertes Männchen. 1. u. 3. Pleopod. Nur Exopoditen vorhanden. (Nach Nilsson-Cantell.)

Abb. 4. *Anapagurus chiroacanthus*. Nicht infiziertes Weibchen. 1. u. 3. Pleopod. Exo- und Endopoditen vorhanden. (Nach Nilsson-Cantell.)

Abb. 5. *Anapagurus chiroacanthus*. Mit *Peltogaster sulcatus* infiziertes Männchen. 1. u. 3. Pleopod. Auftreten von Endopoditen wie bei normalen Weibchen. (Nach Nilsson-Cantell.)

bei Paguriden hervorgerufenen Änderungen der äusseren Geschlechtsmerkmale untersucht. Auch für die Paguriden gilt ähnlich wie für die Brachyuren, dass die vom Parasiten befallenen Weibchen höchstens Rückbildungen, die Männchen hingegen deutliche Umstimmungen erkennen lassen. Bei den infizierten Männchen von *Anapagurus chiroacanthus* kommt es z.B. zur Ausbildung kräftiger Endopoditen

an den Pleopoden des 2-4 Abdominalsegmentes, wie sie den normalen Weibchen eigen sind, bei nichtinfizierten Männchen aber ganz fehlen (vgl. Abbildungen 3-5). Ähnlich liegen die Verhältnisse bei *Eupagurus cuanensis*.

Giard (1887) machte fernerhin Mitteilungen über die Kastration bei *Gebia stellata*, verschiedenen Garneelen (*Palaemon*, *Hippolyte*), die teils durch Rhizocephalen, teils durch schmarotzende Isopoden (Bopyriden) herbeigeführt wird.

**Würmer.** Ueber parasitäre Kastration und ihre Folgeerscheinung bei *Lumbricus herculeus* liegen Beobachtungen von Sollas (1911) vor (s. unten S. 278). Auch bei der Nemertine *Lineus obscurus* und der Planarie *Leptoplana* ist parasitäre Kastration festgestellt worden (Giard, 1888), doch kommt es hierbei zu keiner Auswirkung auf äussere Geschlechtsmerkmale. Das gleiche gilt für verschiedene parasitär kastrierte

**Mollusken und Echinodermen.** Genannt seien hier nur die Schnecken *Paludina*, *Limnaea* und *Planorbis* und der Schlangensterne *Amphiura*. Alle diese Fälle sind in der ausführlichen Arbeit von Wheeler (1910) zusammengestellt.

**Insekten.** Parasitäre Kastration kommt unter den Insekten vor allem bei *aculeaten Hymenopteren* (vor allem *Polistes* und *Andrena*) vor, dann aber auch bei Zikaden. Bei den *aculeaten* Hymenopteren wird sie durch parasitische Strepsipteren, in erster Linie *Xenos* und *Stylops*, bewirkt. Die umfangreiche Literatur über dieses Gebiet ist bei Pierce (1909, 1918) zusammengestellt. Ausserdem sei auf die knappe Darstellung von Ulrich (1927) hingewiesen. Nach den Angaben von Wheeler (1910) u.a. werden durch *Xenos* am *Polistes*-Körper zwar Veränderungen verschiedenster Art bewirkt, eine deutliche Beeinflussung oder gar Umstimmung der sekundären Geschlechtsmerkmale ist jedoch nicht zu beobachten. Anders liegen die Verhältnisse bei *stylopisierten Andrena*. Die Beobachtungen von Pérez (1886), die allerdings von verschiedenen Seiten (z.B. Perkins, 1892, 1918) eine gewisse Einschränkung erfahren haben, weisen auf einen Zusammenhang zwischen Stylopisierung und Ausprägung sekundärer Geschlechtsmerkmale hin. Die Ergebnisse der Untersuchungen von Pérez sind in Tabelle 4 zusammengestellt.

Tabelle 4. *Andrena* (Vergleich zwischen normalen und stylopisierten Tieren).

	Clypeusfarbe	Tibia	Haarkranz am 5. Abd.-Sterniten
Normales Männchen	Gelb	Ohne Differenzierungen	Fehlt
Normales Weibchen	Schwarz	Mit Einrichtung zum Pollensammeln	Vorhanden
Stylopisiertes Männchen	Gelbe Farbe stark vermindert	Zuweilen Auftreten weibchenähnlicher Differenzierungen	Tritt zuweilen auf
Stylopisiertes Weibchen	Auftreten gelber Flecke	Häufig Wegfall der Einrichtung zum Pollensammeln	Fehlt oftmals

Während der Wegfall von Organteilen oder eine Reduktion z.B. des Stachel- und Copulationsapparates, ebenso wie die Herabsetzung der Lebensfrische ganz

allgemeine Folgeerscheinungen des Parasitismus sind, muss man wohl die an der Tibia auftretenden Veränderungen, vor allem aber die Umstimmung der Kopfschild- (Clypeus-) zeichnung auf eine Störung im Gleichgewicht der Sexualhormone zurückführen (s. Abbildung 6).

Wesentlich scheint mir in unserem Zusammenhang die von mehreren Autoren beschriebene Tatsache zu sein, dass das Auftreten solcherlei Merkmalsumstimmungen nicht durch eine völlige Zerstörung der Keimdrüsen selbst bedingt sein muss. Die Parasiten nähren sich ja in der Hauptsache vom Fettkörper ihrer Wirtstiere. Zweifellos erinnert dies an die von Courrier an parasitär kastrierten Krebsen gemachten Beobachtungen (s. S. 273).

Eine ganz eigenartige Wirkung der parasitären Kastration teilt Buchner (1925) von Zikaden mit. Er beschreibt 3 Weibchen von *Euacanthus*, deren Abdomen fast gänzlich von einer parasitischen Dipterenlarve (wahrscheinlich *Pipunculus*) eingenommen wurde. Die *Euacanthus*weibchen besitzen im Gegensatz zu den Männchen

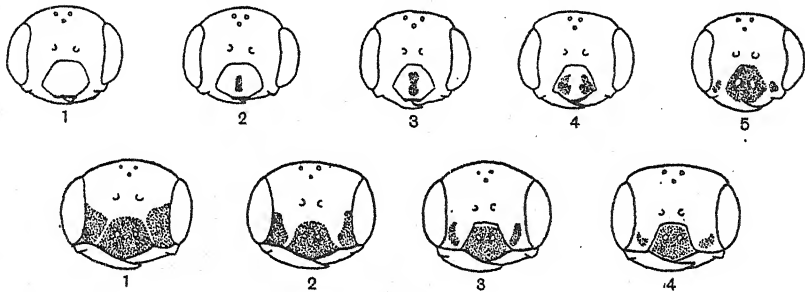


Abb. 6. Veränderungen der Clypeus-Zeichnung bei stylipisierten *Andrena labialis*.—Die punktierten Flecke entsprechen den gelben Färbungen des Clypeus. Obere Reihe: Köpfe weiblicher Bienen. Untere Reihe: Köpfe männlicher Bienen. Fig. 1 in jeder Reihe zeigt Köpfe normaler Tiere. Fig. 2–4 (5) Färbungsänderungen in verschiedener Stärke. (Nach Pérez aus Ulrich.)

an ihren Mycetomen (= Symbiontenbehältern) sogenannte Infektionshügel, aus denen zur Zeit der Geschlechtsreife in bestimmter Weise umgewandelte Symbionten (sog. Infektionsformen) ins Blut übertreten. Bei den parasitär kastrierten Weibchen, die keine Spur ihrer Keimlager mehr aufzuweisen hatten, waren zwar die Mycetome durchaus normal entwickelt, eine durchgreifende Allgemeinschädigung des Wirtes ist also nicht anzunehmen. Die Infektionshügel aber waren ebenso wie die symbiontischen Infektionsformen nur ganz schwach angedeutet.

Aehnliches beschreibt Buchner bei einem *Solenocephalus*weibchen. Wesentlich scheint noch folgender Befund an einer Strongylocephalenlarve, einer Form, deren Mycetome einen ausgeprägten Sexualdimorphismus aufweisen: "Eine parasiten-behaftete Larve enthält ein ganz stehengebliebenes Ovar, das dem Mycetom dort dicht anliegt, wo sich der Infektionshügel bilden sollte. Von seinen Eigentümlichkeiten ist aber keine Spur zu sehen. Gleichalte Larven nahestehender Arten haben die üblichen Prozesse in diesem Alter bestimmt schon eingeleitet."

Wir hätten nach alledem im Infektionshügel des Mycetoms und in der sog. Infektionsform der Symbionten ein sekundäres weibliches Geschlechtsmerkmal zu

erblicken, das dem Männchen fehlt und zu dessen Ausbildung vermutlich "spezifisch-weibliche Reizstoffe" (Buchner, 1925) notwendig sind.

Die hier aufgezählten Fälle sind bei Insekten die einzigen, wo parasitäre Kastration eine direkte, wahrscheinlich hormonale Abhängigkeit der sekundären Sexualcharaktere von den Keimdrüsen an den Tag legt. In den zahlreichen anderen Fällen, in denen Insektenkeimdrüsen von Parasiten zerstört werden (z.B. *Forficula*, Termiten, Scarabaeiden, Nashornkäfer usw.) kommt es höchstens zu Rückbildungen, die durch Stoffwechseländerungen erklärt werden müssen, nicht aber zur Umstimmung von Geschlechtsmerkmalen. Auch in diesem Zusammenhang muss auf die schon genannte Arbeit von Wheeler (1910) hingewiesen werden.

#### WIRKUNGEN EXPERIMENTELLER KASTRATION.

Die zahlreichen Kastrationsversuche, die an Wirbellosen (Würmern, Krebsen, Insekten) vorgenommen wurden, dienten entweder Regenerationsstudien oder suchten die Frage zu beantworten, ob die Ausprägung der äusseren Geschlechtsmerkmale von den Keimdrüsen abhängig ist. Während die Regenerationserscheinungen in unserem Zusammenhang nur von mittelbarer Bedeutung sind, ist der zweite Punkt—die Beziehung zwischen primären und sekundären Geschlechtscharakteren—für uns von besonderem Interesse. Denn wenn eine Abhängigkeit zwischen Gonaden und äusseren Geschlechtsmerkmalen besteht, ist die Annahme weitgehend berechtigt, dass diese Abhängigkeit durch die Vermittlung von Geschlechtshormonen zustande kommt.

*Würmer.* Bezüglich der Bedeutung der Keimdrüsen für die Ausbildung äusserer Geschlechtscharaktere bei Würmern ist ein einziger Fall untersucht und zwar *Lumbricus herculeus* (Harms, 1912). Als äusseres Geschlechtsmerkmal ist der Sattel (Clitellum) anzusehen. Er liegt als deutlich sichtbare Hautverdickung zwischen dem 32. u. 37. Segment. Das Clitellum ist—nach Harms—als ein typisch *cyklisches* Geschlechtsorgan aufzufassen, da es nur während der Geschlechtsperiode seine volle Ausbildung zeigt. Es hat 2 Aufgaben zu erfüllen: einmal bewirken seine Sekrete ein enges Aneinanderschmiegen der Tiere bei der Begattung; zum andern liefert der Sattel bei der Eiablage die Kokonhüllen.

Bekanntlich sind die Regenwürmer Zwitter. Harms legte sich nun die Frage vor: ist die Ausbildung eines Clitellum von den männlichen oder von den weiblichen Keimdrüsen abhängig? Das Ergebnis seiner Versuche war folgendes: Entfernung der Ovarien ändert nichts am cyklischen Auftreten des Clitellum, Hodenexstirpation hingegen bewirkt Schwund des Clitellum. Selbst ein Jahr nach der Operation war trotz guter Ernährung der Versuchstiere keine Sattelbildung zu beobachten. Es darf somit für möglich gehalten werden, dass bei *Lumbricus herculeus* die Entwicklung des Clitellum in hormonaler Abhängigkeit von den männlichen Keimdrüsen steht. Zu der Frage, ob das postulierte inkretorische Organ mit den Keimdrüsen selbst identisch ist oder nicht, kann im vorliegenden Fall deshalb nichts geäußert werden, weil Harms die Kastration durch völlige Entfernung sämtlicher männlicher Geschlechtssegmente bewerkstelligte.

Mit Recht sieht Harms in folgender von Sollas (1911) mitgeteilten Beobachtung

eine Bestätigung seiner Versuche: Sollas fand mehrere freilebende *Lumbricus herculeus*, die kein Clitellum aufwiesen. Die nähere Untersuchung ergab, dass die Hoden der betreffenden Tiere durch Parasiten zerstört waren, während sich die Ovarien als völlig unverletzt erwiesen.

Neuerdings hat Avel (1928) Untersuchungen über die Ausbildung der Gonaden und des Clitellum vorgenommen. Seine Versuchstiere waren *Allolobophora terrestris* und *caliginosa*. Auch Avel rechnet mit hormonalen Wirkungen, betont aber ausdrücklich, dass eine strenge Beziehung zwischen der Ernährung und der Entwicklung der Genitalorgane bestehe. Allerdings hat er in seiner letzten Veröffentlichung (1928), ohne die Harmsschen Versuche eigens zu widerlegen, jede Abhängigkeit der sekundären Sexualcharaktere von den Keimlagern in Abrede gestellt. Es wäre sehr wünschenswert, wenn dieser Zwiespalt durch erneute Nachuntersuchungen eine endgültige Klärung fände.

**Krebse.** Als wir die Folgeerscheinungen parasitärer Kastration bei Krebsen durch das Fehlen von Geschlechtshormonen zu erklären versuchten, mussten wir immer einen sehr beachtlichen unbekannten Faktor in Rechnung setzen, nämlich die allgemeinen, vor allem den Stoffwechsel beeinflussenden Wirkungen des Parasiten. Es ist darum sehr zu begrüßen, dass in neuester Zeit an Krebsen sehr exakte Kastrationsversuche vorgenommen wurden.

Sowohl Vandel (1924) als auch Legueux (1924) beschäftigten sich mit der Frage, ob bei Krebsen (Landasseln, bezw. Gammariden) die Ausbildung eines Brutsackes vom Zustand des Ovariums abhängt. Die Antwort darauf hat Haemmerli-Boveri (1926) in einer sehr gründlichen Arbeit gegeben, die betitelt ist: "Die Determination der sekundären Geschlechtsmerkmale (Brutsackbildung) der weiblichen Wasserassel durch das Ovar." Der Haemmerli-Boverischen Veröffentlichung sei das Folgende entnommen.

Die weibliche Wasserassel (*Asellus aquaticus*) zeigt einen streng geregelten Geschlechtszyklus. Seine Hauptkomponenten sind: Eientwicklung in den Ovarien, Brutsackbildung, Eiablage und Brutpflege; Häutungsvorgänge treten gesetzmässig hinzu. Beim normalen Tier laufen diese Geschehnisse folgendermassen ab:

(a) bei der *Eientwicklung* sind in der Hauptsache 4 aufeinanderfolgende Stadien zu unterscheiden, die in Tabelle 5 kurz gekennzeichnet sind.

Tabelle 5.

Stadium	Zustand der Ovarien	Lage der Eizellen	Beschaffenheit der Eier
I	Dünn u. schlauchförmig	Nur im Gebiet des sog. Randstreifens vorhanden	Grosser Kern mit grossem Nucleolus
II	Teilweise gefüllte Schläuche	Eier treten aus dem Randstreifen heraus u. erfüllen teilweise den Ovarialschlauch	Dotterreich, von mittlerer Grösse, Kern sichtbar
III	Pralle Schläuche	Die Eier erfüllen die ganzen Ovarien	Eier platten sich gegenseitig ab, Kern schlecht oder nicht sichtbar
IV	Die Rückbildung beginnt	Eiablage in den Brutsack	—



An das Stadium III schliesst sich die Ausstossung der Eier in den Brutsack an, worauf nach Ablauf der Embryonalentwicklung das Ausschlüpfen der jungen Asseln erfolgt.

(b) Die *Brutsackbildung* ist ebenso wie die Eibildung unabhängig davon, ob eine Befruchtung erfolgte oder nicht. Der Brutsack dient bekanntlich zur Aufnahme der aus dem Ovarium austretenden Eier. Der Brutsack wird "durch 8 Chitinlamellen gebildet, die im Bereich des Thorax an den vorderen 4 Beinpaaren ansetzen." Diese Brutlamellen weisen zwei ganz verschiedene, sich cyklisch abwechselnde Ausbildungsformen auf: sie sind entweder schmale, ziemlich einzeln liegende Leistchen oder aber stark verbreiterte Lamellen, die sich gegenseitig an ihren Rändern überdecken und so eine Art Sack bilden. Diese verschiedenen Ausprägungen des Brutsacks stehen im engsten Zusammenhang mit

(c) *Häutungsvorgängen*, die sich nach van Emden (1922) und Haemmerli-Boveri folgendermassen voneinander unterscheiden: bei der *Parturialhäutung* wird der Brutsack gebildet, bei der nach dem Ausschlüpfen der Embryonen stattfindenden *Zwischenhäutung* geht er wieder verloren. Die sog. *Normalhäutungen* dienen lediglich Wachstumsvorgängen, haben aber mit dem Brutsack nichts zu tun.

Wie die im vorstehenden geschilderten Geschehnisse ineinandergreifen, ist aus Tabelle 6 zu ersehen, in der jede wagerechte Kolumne gleichzeitige Vorgänge kundgibt.

Tabelle 6.

Zustand des Brutsacks	Häutungsvorgänge	Zustand der Gonaden u. Eier (vgl. Tabelle 5)
Brutsack ausgebildet	Parturialhäutung eben vollendet	Stadium IV beginnt
Brutsack voll sich entwickelnder Eier	Zeit zwischen Parturial- u. Zwischenhäutung	Stadium I und II
Nur kleine Brutlamellen, kein Brutsack	Zwischenhäutung eben vollendet	Stadium II und III

Dass die beschriebenen cyklischen Geschlechtsgeschichte Vorgänge miteinander in Beziehung stehen, ist klar. Es fragt sich nun: inwieweit hängen sie von den Keimdrüsen ab. Haemmerli-Boveri nahm nun Kastrationsversuche durch Radiumbestrahlung vor. Zwar wurden die ganzen Körper der Asselweibchen den Radiumstrahlen ausgesetzt, es zeigte sich aber, dass kein Organ ausser den Ovarien durch diese Behandlung angegriffen wurde.

Die durchschnittliche Bestrahlungsdosis betrug 20 mg RaBr<sub>2</sub> (3 × 10 Stunden innerhalb 5–6 Tagen). Die Bestrahlung wirkte nach einer mehrtägigen Latenzzeit eine weitgehende Degeneration der Ovarien aus: sie enthalten je nach Dauer der Einwirkung keine oder nur wenige Eier, die Ovarialstreifen sind nicht mehr zu erkennen, die Zellkerne verkümmern. Das Wesentliche sind für uns die Folgeerscheinungen, die sich auf die Brutsackbildung beziehen. Hier sind zweierlei Wirkungen zu unterscheiden: erfolgt die erste Bestrahlung etwa 8 Tage vor der Parturialhäutung, also der Brutsackbildung, dann wird der Brutsack doch noch gebildet und—obwohl

das Ovar vollständig zerstört ist—die normale Anzahl von Tagen beibehalten, um bei der Zwischenhäutung verloren zu gehen. Die nun folgende Häutung aber ist nun nicht, wie beim unbestrahlten geschlechtsreifen Weibchen, eine von Brutsackbildung begleitete Parturialhäutung, sondern eine Normalhäutung, bei der kein Brutsack auftritt. Selbst bei mehrmonatlicher Beobachtung bestrahlter Weibchen treten zwar Normalhäutungen auf, nie mehr aber Parturialhäutungen: *die Fähigkeit der Brutsackbildung ist mit der Zerstörung der Ovarien verloren gegangen.*

Dabei ist im Auge zu behalten, dass das Fehlen des Brutsacks nicht auf einer direkten Bestrahlung dieses sekundären Geschlechtsorgans beruhen kann. Tritt er ja doch—wie aus dem vorstehenden zu ersehen ist—noch einmal nach oder sogar während der Bestrahlungsperiode auf, um dann aber für immer zu verschwinden.

Bemerkenswert ist noch der Befund, dass in der Jugend (4–5 mm Länge) bestrahlte Asselweibchen zwar ein ganz normales, von Häutungen begleitetes Wachstum zeigen, aber nur die schmalen Brutlamellen, niemals aber Brutsackbildung aufweisen.

Zusammenfassend sei aus den Untersuchungen von Haemmerli-Boveri das folgende festgehalten:

Beim normalen geschlechtsreifen Asselweibchen vollzieht sich folgender Zyklus: Parturialhäutung—Brutsackperiode—Zwischenhäutung—kurze brutsacklose Periode—Parturialhäutung—Brutsackperiode usw.

Nach Zerstörung der Ovarien durch Radiumbestrahlung ändert sich der Zyklus folgendermassen: Brutsackperiode—Zwischenhäutung mit endgültigem Verlust dieses Geschlechtsmerkmals—Reihe von Normalhäutungen, die nur dem Wachstum dienen.

Während die *kleinen* Brutlamellen von *Asellus*weibchen wahrscheinlich nicht von den Gonaden abhängen, ist der Brutsack als sekundäres Geschlechtsmerkmal anzusehen, dessen Vorhandensein die inkretorische Tätigkeit eines funktionsfähigen Ovariums voraussetzt. Diese Tatsachen können in Beziehung gesetzt werden zu Beobachtungen von Sexton und Huxley (1921) an Intersexen von *Gammarus chevreuxi*. Auch diese Forscher fanden neben sekundären Geschlechtsmerkmalen, die der Keimdrüsenwirkung unterliegen, Bildungen (Chitinstrukturen), die ohne Rücksicht auf die innersekretorischen Zustandsänderungen des Intersexes erhalten bleiben.

*Insekten.* So sehr die an Krebsen beobachteten Kastrationswirkungen einen Zusammenhang zwischen primären und sekundären Geschlechtsmerkmalen und damit auch das Vorhandensein von Geschlechtshormonen vermuten lassen, so wenig scheinen dies zahlreiche gleichartige Versuche an Insekten darzutun.

Oudemans (1898) war der erste, der, angeregt durch Beobachtungen an Gynandromorphen, Kastrationsversuche bei Schwammspinnerraupen (*Ocnèria* (*Lymantria*) *dispar*) vornahm. Diese Form schien wegen ihres stark ausgeprägten Geschlechtsdimorphismus—natürlich bei der Imago—besonders zu solchen Versuchen geeignet. Der Geschlechtsdimorphismus äussert sich in Grösse, Farbe, Antennenform usw. Oudemans entfernte die dorsalwärts im achten Körpersegment der Raupen liegenden, durch ihre gelbliche Farbe leicht erkennbaren Keimdrüsen sowohl bei männlichen als auch bei weiblichen Tieren. Die Kastration wurde ein- oder beidseitig vorgenommen und zwar eine beträchtliche Zeit vor der Verpuppung. Es ist klar, dass die

Kastration auf einem möglichst frühen Stadium ausgeführt werden musste, um eine etwaige hormonale Keimdrüsenwirkung weitgehend hintanzuhalten. Das Ergebnis der Oudemanschen Versuche ist völlig eindeutig. Keines der über 30 Tiere, die Operation und Verpuppung überstanden, zeigte auch nur die geringste Abweichung von normalen männlichen, bzw. weiblichen Habitus. Und noch mehr: selbst beiderseits kastrierte Falter, deren anatomische Untersuchung keine Spur von Keimdrüsen mehr erkennen liess, legten einen völlig unveränderten Geschlechtstrieb an den Tag: die Männchen vollzogen die Copula, ohne dass Spermatozoen vorhanden gewesen wären und die Weibchen setzten in durchaus normaler Weise die Wolle ihres Hinterleibes ab, obwohl sie natürlich keine Eier in das Wollkissen ablegen konnten. Die Kastration hatte also weder in morphologischer noch in physiologischer Beziehung die sekundären Geschlechtseigentümlichkeiten irgendwie beeinflusst.

Mit dem Ergebnis der Oudemanschen Versuche stimmen die von Kellogg an *Bombyx mori* gewonnenen Befunde völlig überein.

Die Kastrationsversuche von Oudemans am Schwammspinner hat Meisenheimer (1907-8) wiederholt und weiter ausgebaut. Einmal war sein Tiermaterial bedeutend grösser: aus 600 operierten Raupen gelang die Aufzucht von 186 Faltern, ausserdem wurden nicht nur die Keimdrüsen selbst, sondern auch die Anlagen der Anhangsdrüsen und der Ausführgänge entfernt. Eine ganz wesentliche Erweiterung bestand aber darin, dass in einer grösseren Versuchsreihe kastrierten Tieren die Gonaden des jeweils entgegengesetzten Geschlechts eingepflanzt wurden.

Alle diese Eingriffe waren in einer grossen Zahl von Fällen von Erfolg begleitet: in den kastrierten Tieren traten keine Keimdrüsenregenerate auf und die in ganz embryonalem Zustand transplantierten Gonaden (vor allem Ovarien) wuchsen im Körper des entgegengesetzten Geschlechts heran. Die Ovarien verwuchsen sogar mit den männlichen Ausführgängen.

Das Ergebnis dieser gut gelungenen Versuche stimmte vollständig mit den Resultaten von Oudemans überein: Kastraten und "künstlich erzeugte innere Zwitter" zeigten keinerlei Änderung in Körperform, Flügelfärbung und Fühlerbildung. Es hatten also beispielsweise Falter durchaus männliches Aussehen, trotzdem sie funktionsfähige Ovarien im Leibe trugen.

Eine einzige Einwirkung der Operationen auf äussere Merkmale hat Meisenheimer festzustellen vermocht: es ist die Variationsbreite der Flügelfärbung bei den operierten Tieren weitaus grösser, als bei den normalen Faltern, und zwar so, dass die dunkelsten Varianten der operierten Weibchen der Färbung normaler Männchen bedeutend näher kommen, als dies bei den dunkelsten Varianten nicht operierter Weibchen jemals zu beobachten ist.

Eine bedeutsame Erweiterung des Experimentes brachte Meisenheimer späterhin (1908) dadurch, dass er bei kastrierten *Ocneriaraupen* und bei Kastratenmännchen, die Ovarien-Transplantate erhalten hatten, nach der dritten Häutung die Flügelanlagen einer Seite entfernte und nun die Ausbildung der Flügelregenerate beobachtete. Der Sinn dieser Massnahme ist der: man könnte annehmen, dass die Kastration, bzw. Gonadentransplantation, zu spät ausgeführt wurde, dass also durch hormonale, von der Keimdrüse ausgehende Einflüsse, die Geschlechtsbestimmung der Flügel-

anlage bereits vollzogen ist. Dieser Fehler wird natürlich vermieden, wenn man die Ausgestaltung von Flügelregeneraten beobachtet, die sich vom ersten Anfang an unter dem möglichen Einfluss einer gegengeschlechtlichen Keimdrüse befunden haben. Aber auch durch diese feinsinnige Ausgestaltung der Versuchsweise konnte eine Gonadenabhängigkeit der äusseren Geschlechtsmerkmale in keinem Falle erwiesen werden.

Es ist das Verdienst Kopeč's (1908, 1910, 1911, 1913, 1924) an einem ungeheuren Material von Raupenarten und -individuen und mit den verschiedensten Versuchsanordnungen die ganze Frage durchgearbeitet zu haben. Kopeč beschränkte sich nicht nur auf einfache Kastration und Transplantation, sondern nahm auch Transfusionen von Blut und Gonadenplasma in vielfältigster Weise vor. Zudem hat er zur Steigerung der Transplantatwirkungen nicht nur eine oder zwei, sondern oftmals mehrere Gonaden einem gegengeschlechtlichen Tier eingepflanzt. Hierbei wurden nicht nur im Abdomen, sondern auch im Thorax Keimdrüsen zur Entwicklung gebracht. Die zahlreichen, vor allem auch histologisch wertvollen Einzelergebnisse der Kopeč'schen Untersuchungen brauchen hier nicht besprochen zu werden. Letzten Endes ergaben sie alle die Tatsache, dass die äusseren Geschlechtsmerkmale, vor allem die Flügel, ebenso wenig wie das Verhalten der Falter (Begattungstrieb der Männchen usw.) einer Gonadenhörigkeit unterliegen (vgl. hierzu auch Klatt, 1919, 1920).

Ergänzend sei hier auf die von Prell (1914) an Eichenspinnerraupe (*Lasiocampa quercus*) vorgenommenen Kastrationsversuche hingewiesen. In diesem Fall wurden mit besonderer Gründlichkeit die Einzelheiten des sexualdimorphen Antennenbaues untersucht. Aber auch hier das gleiche: Kastraten unterscheiden sich nicht von normalen Faltern.

Weiterhin hat Prell (1915) am Grasspinner (*Cosmotriche potatoria*) Kastrationen und Gonadentransplantationen ausgeführt. Dieses Versuchstier schien deswegen besonders geeignet, weil es in den Wärmeversuchen von Frings (1908, 1912) und Standfuss (1899, 1913) eine weitgehende Labilität des sexualen Färbungsdimorphismus erwiesen hatte. Eine Beeinflussung der Flügelfarbe durch Kastration oder Transplantation schien daher hier am leichtesten zu erzielen. Und in der Tat hat Prell bei *Cosmotrichemännchen* eine durch die Operation bedingte Veränderung der Haemolymph und der Flügelfarbe beobachten können. Während für den ersten Faktor wahrscheinlich Stoffwechselvorgänge verantwortlich gemacht werden können, dürfte es sich für die Farbänderung um einen Einfluss der Gonaden handeln. Die kastrierten und mehr noch die mit Ovarien versehenen Männchen zeigten in ihrer Flügelfarbe eine unverkennbare Annäherung an den weiblichen Habitus.

Im Gegensatz zu den an Schmetterlingen so zahlreich durchgeführten Kastrationsversuchen sind andere Insekten bisher nur selten in den Kreis der Betrachtung einbezogen worden. Lediglich an *Gryllus campestris* hat Regen (1909, 1910) Kastrationsversuche ausgeführt. Männlichen wie weiblichen Grillen wurden im vorletzten oder letzten Larvenstadium die Keimdrüsen entfernt. Die Spermatophorendrüsen wurden den Tieren belassen. Leider hat Regen die exstirpierten Testikel, bezw. Ovarien wieder an seine Versuchstiere verfüttert. Da dies wohl nicht in allen Fällen

geschah, kann den Versuchen doch eine gewisse Beweiskraft zugesprochen werden. Die kastrierten Grillen benahmten sich wie normale Tiere: kastrierte Männchen und Weibchen bewohnten, als sie geschlechtsreif geworden waren, ein gemeinsames Erdloch. Die männlichen Kastraten zirpten und suchten ihre Spermatophorenhüllen, die natürlich keine Samenfäden enthielten, anzubringen. Die Weibchen bohrten wie bei der Eiablage die Legeröhre in die Erde.

Von wesentlicher Bedeutung sind die Versuche von Dewitz (1908, 1912), Steche (1912) und vor allem von Geyer (1913). Zumal dem letztgenannten Autor gelang es unter Anwendung der verschiedensten Methoden nachzuweisen, dass bei vielen Insekten die beiden Geschlechter eine deutlich sexuell-spezifische Haemolymph besitzen. Diese Verschiedenheit der Haemolymph kann weder durch Kastration, Gonadentransplantation oder Bluttransfusion beeinflusst werden. Serologisch (Precipitinmethode) liessen sich allerdings differente Eiweisskörper bei Männchen und Weibchen feststellen. Nach Geyer (S. 487) u.a. bestünde nur "ein spezifischer Einfluss der Geschlechtsdrüsen auf den *Stoffwechsel* (Hormone), der sich aber bei den einzelnen Tiergruppen in verschiedenem Masse geltend macht, je nach Stärke der ab ovo gegebenen sexuellen Differenzierungen des Somas."

Wenn wir aus den beschriebenen Versuchen an Insekten (vgl. Kammerer, 1912) alles herausziehen wollen, was irgendwie zu Gunsten einer hormonalen Beeinflussung der äusseren Geschlechtsmerkmale durch die Keimdrüsen sprechen könnte, so ist dies nicht sehr viel. Eine Ausnahme bilden nur die Befunde von Pérez (parasitische Kastration von *Andrena*) und die Färbungsänderungen, die Meisenheimer und Prell in einigen Fällen beobachteten. Im allgemeinen muss wohl bis heute für die Insekten gelten, was Meisenheimer (1907-8) sagt: "Die Bestimmung der äusseren Form, soweit sie mit den sekundären Geschlechtsmerkmalen zusammenhängt, muss also in der Entwicklung viel weiter zurückliegen, als das erste sichtbare Auftreten der mit dieser äusseren Form in Beziehung stehenden Anlagen, sie liegt wahrscheinlich ebensoweit zurück, wie die Bestimmung der Geschlechtsdrüsen selbst." Diese Tatsache stellt—falls sie sich auch weiterhin bewahrheiten sollte—die Insekten in schärfsten und auffälligsten Gegensatz zu den Crustaceen und zu den Wirbeltieren. Dass die eigentlichen Keimdrüsen für die Ausprägung der sekundären Sexualcharaktere bei den Insekten ohne Bedeutung sind, das ist durch die unzähligen Raupenversuche wohl sicher bewiesen. Aber könnten nicht, wie Harms bereits 1914 (S. 147) ausführt, "andere Gewebsgruppen in dem Insektenkörper für die Korrelationen eine Rolle spielen, so z.B. die Oenocyten oder der Fettkörper"? "Die Blut- und Keimplasmainjektionen von Kopeć können nicht als Beweise herangezogen werden. Sie konnten... eine vorübergehende biologische Umstimmung bedingen."

Eine ähnliche Meinung vertritt v. Buddenbrock (1928, S. 671): "Soviel ich sehe, wird aus diesen Versuchen von allen Autoren der Schluss gezogen, dass bei den Insekten die sekundären Geschlechtscharaktere von vornherein, d.h. von der Befruchtung ab, mit dem Geschlecht zugleich determiniert sind. Dieser Schluss ist aber nicht absolut zwingend. Es ist entsprechend der Vorstellungen Courriers über die Krebse durchaus möglich, dass die sekundären Geschlechtscharaktere auch der Insekten der Beeinflussung durch eine innersekretorische Drüse unterliegen, die von

den Keimlagern räumlich getrennt ist, und deren experimentelle Ausschaltung bisher nicht gelang."

Diese Hinweise erhalten m.E. ein besonderes Gewicht durch das, was bei der parasitären Kastration von *Andrena* und der Zikade *Strongylocephalus* zu beobachten war. Ebenso wie bei den Krebsen Courriers ist bei den genannten Insekten eine Umstimmung gewisser Geschlechtsmerkmale zu beobachten, ohne dass dafür der Verlust der Keimdrüsen haftbar gemacht werden konnte. In beiden Fällen muss man also möglicherweise eine Schädigung an einem von den Keimdrüsen getrennten inkretorischen Organ annehmen.

#### ANHANG: INKRETORISCHE EINFLÜSSE DEGENERIERENDER EIER BEI HYMENOPTEREN-ARBEITERINNEN.

Die Arbeiterinnen von *Formica rufa* und *Camponotus ligniperda* bilden in ihren Eischläuchen wenige, aber unverhältnismässig grosse Eier aus, die nicht abgelegt, sondern bis auf einen Restkörper resorbiert werden. Weyer (1927 u. 1928), der die Keimdrüsenverhältnisse bei Hymenopteren-Arbeiterinnen aufs genaueste untersucht hat, weist auf die Möglichkeit hin, dass die resorbierten Eistoffe auf das Soma gleichsam inkretorisch wirken. Wahrscheinlich ist die bei den Arbeiterinnen im Gegensatz zu den Königinnen gesteigerte Vitalität auf diese resorbierten Stoffe zurückzuführen. Einen ähnlichen Hinweis finden wir bei Harms (1926) für die Hummelarbeiterin. Ob es sich hierbei um reine Stoffwechselvorgänge oder aber tatsächlich um hormonale Geschehnisse handelt, kann natürlich nur durch entsprechende Versuche entschieden werden.

#### DAS NEBENNIERENRINDEN-ÄHNLICHE ORGAN VON PHYSCOSOMA.

Die einzige Mitteilung über ein Organ eines Wirbellosen, dass zum Interrenalorgan der Wirbeltiere in Vergleich gesetzt werden kann, verdanken wir Harms (1919). An den paarigen Nephridialschläuchen von *Physcosoma*, einem zu den Gephyreen gehörigen Wurm, entdeckte Harms eine zwischen Peritoneal- und Nierenepithel liegende Gruppe polygonaler Zellen, die er als *Internephridialorgan* bezeichnet. (Vgl. Abbildung 7.) Junge Internephridialzellen enthalten noch keine Granula. Bei fortschreitender Reife jedoch treten aus dem Kern Körnchen aus, die sich stark mit Safranin färben. Dieses chromatische Kernsekret erfüllt das Zellplasma, verliert aber allmählich seine starke Färbbarkeit mit Safranin. Mit aller Deutlichkeit konnte Harms beobachten, dass die gereiften Sekretgranula an der Aussenseite des Internephridialorgans in die Blutflüssigkeit entleert werden. (S. Abb. 8.) "Also eine innere Sekretion, wie man sie sich klarer nicht vorstellen kann." Die verbrauchten Zellen werden von den dem Nierenepithel zugekehrten Zellagen aus regeneriert.

Besonders wertvoll ist es, dass Harms die lebenswichtige Bedeutung des Internephridialorgans durch verschiedene Versuche zu erhärten vermochte: beiderseitige Entfernung der Organe zieht nach 2-5 Tagen den Tod der Tiere nach sich. Belässt man den Tieren jedoch nur ein kleines Stückchen des Internephridialorgans einer Seite, so genügt dies, die operierten Physcosomen am Leben zu erhalten. Ebenso können durch Transplantation eines Internephridialstückchens auf die Körper- oder



Darmwand oder auch durch parabiotische Vereinigung mit einem normalen Tier die tötlichen Folgen beidseitiger Exstirpation aufgehoben werden. Gerade die Tatsache, dass bereits ganz kleine Teile des Internephridialorgans von solch grosser

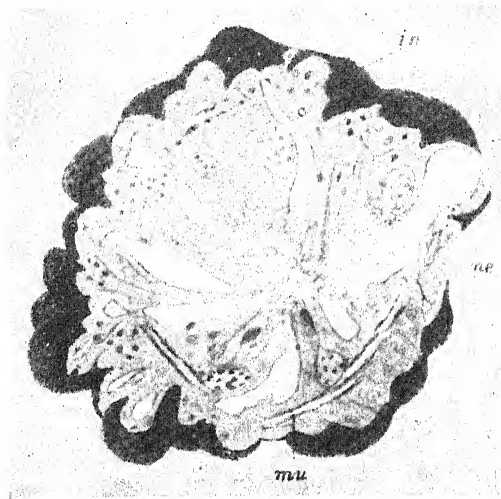


Abb. 7. Schnitt durch den Nephridialschlauch mit Internephridialorgan von *Physcosoma*.  
in = Internephridialorgan; ne = Nierenepithel; mu = Muskulatur. (Nach Harms.)

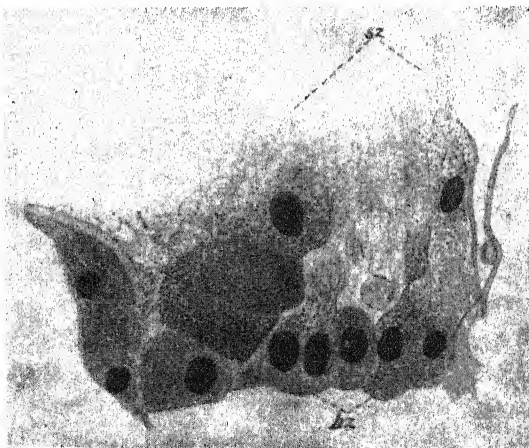


Abb. 8. Zellen des Internephridialorgans von *Physcosoma* im Stadium der Sekretion.  
bz = basale Zellen; sz = in die Blutflüssigkeit sezernierende Zellen. (Nach Harms.)

Wirksamkeit sind, scheint mir ein deutlicher Beweis für die hormonale Natur des Internephridialsekretes zu sein. Ausserdem lässt sich ein morphologisch-histologischer Vergleich zwischen diesem Organ und seiner Sekretbildung und dem Internalstrang der Amphibien und anderer Wirbeltiere weitgehend durchführen.

## INKRETORISCHE VORGÄNGE BEI CEPHALOPODEN.

Vor ganz kurzer Zeit erschien eine Arbeit von Hutchinson (1928), in welcher die Branchialdrüse der Cephalopoden als ein "possible endocrine organ" bezeichnet wird. Allerdings drängen die morphologisch-histologischen Verhältnisse der Branchialdrüse ebenso wie die des Kiemenherzanhangs (Pericardialdrüse) dem Beobachter die Mutmassung auf, dass diese Organe irgendwelche Stoffe in den Blutstrom abgeben. Leider liegen aber über die physiologische Bedeutung dieser Organe bislang noch keine ausreichenden experimentellen Untersuchungen vor. Den Pericardialdrüsen wurde bisher meist exkretorische Funktion zugesprochen.

Von besonderer Bedeutung scheint mir die Tatsache zu sein, auf die Sereni (1928) hinwies: das von Henze (1905, 1913) bei den Cephalopoden nachgewiesene Tyramin (*p*-Oxyphenylaethylamin) wird in den Speicheldrüsen gebildet. Das Tyramin lässt sich aber auch im Blute der Cephalopoden nachweisen. Hierin kann wohl ein ziemlich sicherer Beweis "für das Bestehen einer endokrinen Tätigkeit bei den Cephalopoden" gesehen werden.

## DIE OENOCYTEN.

Bei allen Insekten finden sich—meist in räumlichem Zusammenhang mit dem Fettkörper (s. Abb. 9)—die Oenocyten, auffallend grosse, meist runde Zellen

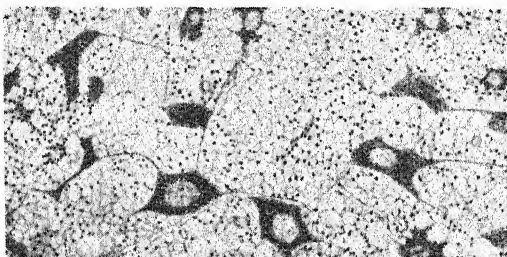


Abb. 9. *Harmonia quadripunctata*. Winterfettkörper mit zahlreichen verzweigten Oenocyten.  
(Nach Kremer.)

von oft weingelber Farbe. Sie sind schon vor 70 Jahren den Forschern aufgefallen, fanden aber ihre erste gründliche und vergleichende Bearbeitung durch Wielowiejski (1886), der ihnen auch den Namen gab. Die Oenocyten sind dann bis in die neueste Zeit in einer bemerkenswerten Reihe von Arbeiten—vor allem in histologischer Hinsicht—aufs genaueste untersucht worden. Es seien hier nur einige wenige Namen genannt: Verson (1891, 1892, 1900, 1911), Wheeler (1892), Koschewnikov (1900), Pérez (1901, 1903), Stendell (1912), Kremer (1917, 1925), Kreuscher (1923) usw. Die ältere Literatur ist bei Pérez, Henneguy (1904), Rössig (1904) und Stendell ausführlich zusammengestellt.

In der Hauptsache sind zwei Arten von Oenocyten zu unterscheiden, die larvalen (grossen) und die imaginalen (kleinen) Oenocyten. Beide dürften ihren Ursprung in der Epidermis nehmen (Verson, Stendell, Kremer), ebenso sind bei beiden sekretorische Vorgänge mit aller Deutlichkeit beobachtet worden. Sonst aber lassen

sich zwischen larvalen und imaginalen Oenocyten eine grosse Anzahl von Unterschieden aufzeigen, die in Tabelle 7 zusammengestellt sein mögen.

Tabelle 7. *Unterschied zwischen den larvalen und imaginalen Oenocyten.*

	Bezeichnung Versions	Grösse	Zeit d. ersten Auftretens	Vermehrung	Lagebeziehung	Verhalten des Kerns
Larvale Oenocyten	Hypostigmatische Drüsenzellen	80-100 $\mu$ (Apis) 180-200 $\mu$ (Ephestia)	In der Intra- ovalperiode	Keine Vermehrung in der extraovalen Periode	Meist in engster Beziehung zu den Tracheen; einzeln oder in Bändern	Im Zusammenhang mit den Sekretions- vorgängen stark verän- derlich (Verästelungen)
Imaginale Oenocyten	Epigastrische Drüsenzellen	30 $\mu$ (Ephestia)	Kurz vor Beginn der Puppenruhe	Massenhafte Vermehrung durch amitotische Teilung während der Puppenruhe (Bombyx) oder in der Geschlechtsperiode (Harmonia)	Keine Beziehungen zu den Tracheen. Oft in Flächen oder Klumpen	Kern stets rundlich, keine Fortsätze

Viel umstritten war die Frage nach der physiologischen Bedeutung der Oenocyten. Die enge Beziehung zwischen larvalen Oenocyten und Tracheen veranlasste Landois (1865) dazu, sie als "Respirationszellen" zu bezeichnen, während P. Schulze (s. Stendell, 1912) die Meinung äusserte, sie könnten für die Häutung der englumigen Tracheen von Bedeutung sein.

Sehr häufig wurden die Oenocyten als "Exkretionszellen ohne Ausführgänge" (Koschewnikov, 1900), also gleichsam als Speicher für Ausscheidungsstoffe angesehen. Vor allem sollten sie in der Zeit der Puppenruhe die Tätigkeit der Malpighischen Gefässe ersetzen. Die Auffassung der Oenocyten als Exkretionsorgane ist wohl häufig auf eine Verwechslung mit den Exkretionszellen bzw. Uratzellen des Fettkörpers zurückzuführen. Eine klare Scheidung zwischen Exkretzellen und Oenocyten vorgenommen zu haben, ist vor allem ein Verdienst von Berlese (1899, 1902), Verson, Stendell, Schnelle (1925) u.a.—Kremer (1925) macht fernerhin auf die scheinbar recht nahen Beziehungen zwischen Oenocyten, Häutungs- und Wehrdrüsen der Coleopteren aufmerksam.

Heutzutage kann es als unbestrittene Tatsache gelten, dass die Oenocyten Drüsenzellen sind, und zwar Drüsen innerer Sekretion, die ihr Sekret unmittelbar in die Blutbahn entleeren. Mit aller Deutlichkeit hat Verson (1911) dies ausgesprochen und gleichzeitig schrieb Anglas (1911): "Aussi les considérons-nous comme des cellules glandulaires (nées de l'hypoderme), et jouant le rôle de glandes à sécrétion interne, mais de glandes dissociées." Nahezu alle späteren Untersucher

dieser Drüsenzellen, wie Stendell, Kremer, Kreuscher, Schnelle kommen zu dem gleichen Schluss.

Eine ausführliche Darstellung der Sekretionsvorgänge verdanken wir—neben Verson—Stendells Untersuchungen an *Ephestia kuehniella*. Seiner Arbeit sei folgendes entnommen (vgl. die Abbildungen 10–14):

Sobald bei der jungen Larve die bleibende Anzahl von Oenocyten ausgebildet ist, treten in den Kernen dieser Drüsenzellen zahlreiche Tröpfchen eines vermutlich zähflüssigen, stark lichtbrechenden, unfärbbaren Sekretes auf (Abb. 10). Die Tröpfchen sammeln sich in Vakuolen, um dann plötzlich aus dem Kerninnern an die Aussenfläche des Kerns, also in das Zellplasma überzutreten (Abb. 11). Die



Abb. 10.



Abb. 11.

Abb. 10. *Ephestia kuehniella*. Larvale Oenocyten. Beginn der Sekretion: Im Oenocytenkern sind Einschlüsse sichtbar. (Nach Stendell.)

Abb. 11. *Ephestia kuehniella*. Larvale Oenocyten. Die Sekrettröpfchen sind aus dem Kern ins Plasma getreten. (Nach Stendell.)

Sekrettröpfchen verteilen sich alsdann überall im Plasma (Abb. 12), sind bald darauf nur noch an der Zellperipherie zu sehen (Abb. 13) und verschwinden schliesslich ganz. „Das Sekret hat also seinen Weg vom Kern durch das Plasma in die Körperhöhle genommen.“ Nach beendigter Sekretion tritt ein—allerdings nur kurzes—Ruhestadium ein: das Zellplasma ist klar, der Kern normal. Dann beginnen die Sekretionserscheinungen von neuem. Bemerkenswert ist dabei, dass die Sekretmissionen von einem zum andern Male „stürmischer“ verlaufen. Dies bewirkt bei Beginn einer Sekretionsperiode ein pralles Aussehen des Kerns, der nach der Entleerung deutlich zusammengezogen und mit Einbuchtungen (s. Abb. 12 u. 13) versehen erscheint. Bei älteren Larven kommt es sogar zu Zersprengungen des Kerns.

Während und nach der Puppenhäutung nimmt die Sekretion ihren Fortgang. Bei der fertigen Puppe aber ist—im Gegensatz zur alten Larve—der Oenocytenkern wieder restauriert: Verästelungen treten nach der Sekretausstossung nicht mehr auf. Gleichzeitig scheint das Sekret selbst eine Wandlung durchgemacht zu haben:

es ist dünnflüssiger geworden und tritt nun nicht mehr im Tropfen, sondern in Bächen aus dem Kern aus. Dadurch entstehen im Oenocytenplasma radiäre,



Abb. 12.



Abb. 13.

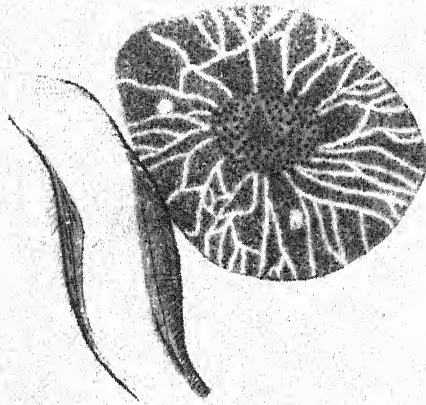


Abb. 14.

Abb. 12. *Ephestia kuehniella*. Larvale Oenocyte. Die Sekretkörner erfüllen das Zellplasma. Der Kern zeigt Kontraktionserscheinungen und Verästelungen. (Nach Stendell.)

Abb. 13. *Ephestia kuehniella*. Larvale Oenocyte. Die Zahl der Sekrettröpfchen nimmt ab. Sie finden sich hauptsächlich an der Zellperipherie. (Nach Stendell.)

Abb. 14. *Dytiscus marginalis*. Ältere larvale Oenocyte. Das Sekret scheint dünnflüssiger zu sein; daher Ausbildung von sog. "Sekretkapillaren." (Nach Kreuscher.)

sich verzweigende Kanäle, bei denen es sich aber kaum um präformierte Bahnen handeln dürfte. Auch Kreuscher (1923) hat diese "Sekretkapillaren" bei den larvalen Oenocyten von *Dytiscus* beobachtet und abgebildet. (Vgl. Abb. 14.)

Hierauf verfallen die grossen larvalen Oenocyten unter chromatolytischen Erscheinungen der Degeneration, nach dem schon vorher—kurz vor dem Einspinnen zur Puppenruhe—die kleinen imaginalen Oenocyten zur Ausbildung gelangt sind. Bei diesen imaginalen Drüsenzellen verlaufen die Sekretionsvorgänge ganz ähnlich wie bei den Larvaloenocyten. Doch sind sie von viel geringerer Heftigkeit und kürzerer Dauer.

So gründlich und lückenlos die Sekretionsvorgänge selbst uns bekannt sind, so geringfügig ist unser Wissen von den *physiologischen Aufgaben*, denen diese inkretorischen Drüsenzellen dienen. Zu dem wenigen, was auf diesem Gebiet bisher rein experimentell gearbeitet wurde, gehören die Fütterungsversuche, die Koschevnikov (1909) in Anlehnung an Kowalewsky (1889) vorgenommen hat. Koschevnikov fütterte Bienen mit flüssigem Futter +  $\text{FeCl}_3$ , tauchte dann kurze Zeit danach Fettkörperstücke seiner Versuchsbienen in Ferrocyankali +  $\text{HCl}$  und stellte fest, dass nur im Innern der Fettzellen, *nicht* aber der Oenocyten, die Berliner-Blaureaktion eintrat. Daraus lässt sich der zweifellos richtige Schluss ziehen, dass die Oenocyten mit der Nahrungsspeicherung nichts zu tun haben. Fernerhin hat Hollande (1914) auf biochemischem Wege den sicheren Nachweis erbracht, dass die Oenocyteneinschlüsse keine Exkretstoffe, sondern Sekretkörner sind.

Sonst liegen über die physiologische Funktion der Oenocyten nur Vermutungen vor. Bedeutsam scheint mir folgende Feststellung Versons zu sein: die Sekretionsvorgänge in den Oenocyten verlaufen—wenigstens bei jungen und mittleren Larven von *Bombyx mori*—synchron mit den Häutungsvorgängen. Ob nun diese beiden Erscheinungen, Oenocytensekretion und Häutung, in unmittelbarer Beziehung (s. unten S. 293) miteinander stehen, oder ob der sich rhythmisch ändernde Stoffwechsel ("Blutstoffwechsel"—Stendell) das letztlich Ausschlaggebende ist, muss einstweilen dahingestellt bleiben. Hinzugefügt sei noch, dass Kremer (1925) die Oenocyten als "Vermittler des intermediären Stoffwechsels bei der Imago" auffasst.

#### INKRETORISCHE VORGÄNGE BEI DER HÄUTUNG UND VERPUPPUNG DER RAUPEN.

Eine Mitwirkung von Hormonen ist wohl am ehesten bei solchen Lebensvorgängen zu erwarten, die in irgend einer Weise den Gesamtorganismus in Mitleidenchaft ziehen. Es sind hier wohl in erster Linie Wachstumsvorgänge zu nennen. Wie stark Wachstums- und Entwicklungsgeschehnisse—unmittelbar oder in engster Beziehung zu Stoffwechselercheinungen—mit der Tätigkeit inkretorischer Organe in Zusammenhang stehen können, wissen wir ja aus der Hormonphysiologie der Wirbeltiere zur Genüge. Es lag darum nahe, Vorgänge wie die Häutung und Verpuppung der Raupen, die ja den ganzen Körper dieser Tiere weitgehend beeinflussen, auf inkretorische Erscheinungen hin zu untersuchen. Die diesbezüglichen Versuche, die Koller (1927) auf Anregung v. Buddenbrocks ausführte, sind noch nicht abgeschlossen. Eine vorläufige Mitteilung darüber, findet sich in v. Buddenbrocks *Grundriss der vergleichenden Physiologie* (1928, S. 698–9).

Die Schwierigkeit und Langwierigkeit dieser Versuche besteht darin, dass man



naturgemäss über eine grosse Zahl von Versuchs- und Kontrolltieren verfügen muss, wobei von jedem der einzeln gehaltenen Tiere das Geburtsdatum und die Häutungsdaten bekannt sein müssen. Zunächst wurde nun versucht, festzustellen, ob das Blut einer sich häutenden bzw. verpuppenden Raupe bei einer anderen Raupe Häutung bzw. Verpuppung hervorrufen kann (Blutübertragung). Natürlich muss der genauebekannte Häutungsrythmus des meist älteren Spendertieres von dem des Empfängertieres entsprechend verschieden sein. Ferner müssen die Durchschnittsdaten für Häutung und Verpuppung an einer grossen Zahl von gleichaltrigen Kontrolltieren, die unter genau gleichen Bedingungen gehalten sind, festgestellt werden.

Blutübertragungen von sich verwandelnden Raupen bzw. Puppen auf jüngere Tiere wurden schon vor geraumer Zeit von Metalnikoff (1907) ausgeführt. Es lag jedoch diesen Versuchen eine ganz andere Fragestellung zu Grunde. Es galt damals zu entscheiden, durch welche Kräfte die bei der Verpuppung auftretende Histolyse der Raupenorgane zustande kommt. Die Körperflüssigkeit junger Puppen hat auf junge Raupen eine deutlich vergiftende Wirkung. Dieses toxische Prinzip des Puppenblutes kann durch halbstündiges Erwärmen auf 60° zerstört werden. Metalnikoff schliesst aus seinen Transfusionsversuchen, dass zum Beginn der Metamorphose im Blute der Insekten bestimmte spezifische Toxine auftreten, die durch Vergiftung bestimmte Organe für die Leukocytenphagocytose gleichsam vorbereiten. Auch Dewitz (1904) sieht in einem Enzym die Ursache für den Eintritt der Metamorphose, sowie für die dabei auftretende Verfärbung der Körperflüssigkeit. Die Häutung bzw. Verpuppung durch entsprechende Blutübertragungen bei geeigneten jüngeren Tieren *auszulösen*, wurde aber m.W. bisher noch nicht versucht. Gehen wir darum zur Besprechung unserer diesbezüglichen Versuche über:

(a) *Häutung*. Bei v. Buddenbrock (1928) findet sich folgende theoretische Darlegung über die Auslösung des Häutungs Vorganges: "Den Anstoss zu diesem ganzen komplizierten Prozess gibt wahrscheinlich das Wachstum, welches, auf den Widerstand des zu engen Cuticularpanzers stossend, das Tier in einen gewissen Reizzustand versetzt. Beantwortet wird dieser Reiz wahrscheinlich durch die Bildung von Hormonen, welche die verschiedenen am Häutungsprozess beteiligten Organe: Hautdrüsen, Nervensystem zu ihrer spezifischen Tätigkeit während der Häutung zwingen." Der Nachweis dieser fraglichen Hormone wurde in den Kollerschen Versuchsreihen hauptsächlich an Raupen von *Sphinx ligustri* erbracht.

Das Herannahen des durchschnittlich 2-3 Tage dauernden Häutungs Vorganges erkennt man bekanntlich daran, dass die Raupe aufhört zu fressen und sich unbeweglich mit leicht aufwärts gekrümmtem Vorderkörper an einem Aestchen festsetzt. Bald darauf beobachtet man die charakteristische Abhebung der Kopf-cuticula. Nun entnimmt man einer solchen in Häutung befindlichen Raupe ca.  $\frac{1}{10}$  ccm Körperflüssigkeit. Als Empfangstier wählt man eine Raupe, deren nächste Häutung frühestens erst in 3 Tagen zu erwarten ist. Diesem Empfänger wird die Körperflüssigkeit des Häutungstieres—natürlich ohne den Darm etc. zu verletzen—eingespritzt. Und nun sind bei einem so behandelten Tier zuweilen

schon nach 5 Stunden, mit ziemlicher Sicherheit aber nach 12 Stunden alle Anzeichen der Häutung zu beobachten. Schädigende Folgen der ganzen Operation, wenigstens für das Empfängertier, sind in der Regel nicht eingetreten.

(b) *Verpuppung*. Versuche über das Hervorrufen frühzeitiger Verpuppung durch Blutübertragung wurden ebenfalls an *Sphinx ligustri*, in der Hauptsache aber an Lindenschwärmerraupe (*Dilina tiliae*) durchgeführt. Das Herannahen der Verpuppung macht sich bei diesen Tieren hauptsächlich durch zwei Erscheinungen bemerkbar: die grüne Körperfarbe wandelt sich im Laufe eines halben Tages in erdfarbenes Braun um und gleichzeitig verlässt die Raupe den Baum (bezw. die Lindenbranche des Zuchtbehälters) und kriecht am Boden umher, um sich dann in die Erde einzugraben. Das Blut eines solchen verpuppungsreifen Tieres vermag nun bei einem anderen, bedeutend jüngeren Tier sämtliche Verpuppungserscheinungen auszulösen. Lindenschwärmerraupe, die—wie eine grosse Zahl gleichaltriger Kontrolltiere zeigten—erst in 9 Tagen zur Verpuppung kamen, begannen 12 Stunden nach der Blutübertragung mit der Verfärbung. Aber nicht nur die Verfärbungserscheinungen wurden auf diese Weise hervorgerufen—dies liesse sich ja schliesslich durch Enzyme erklären—nein: auch das ganze "psychische" Verhalten der Tiere, wenn man so sagen darf, war durch die Blutübertragung verändert: sie verliessen ihre Zweige, krochen am Boden des Zuchtbehälters umher und gruben sich, auf Erde gesetzt, ein. Allerdings muss gesagt werden, dass lange nicht in sämtlichen Fällen die Blutübertragung von dem gewünschten Erfolge gekrönt war. Die Wirkung scheint, soviel bis jetzt zu sehen ist, weniger von dem Zustande des Empfängers als des Spenders abzuhängen. Daraus würde sich der Schluss ergeben, dass die fraglichen im Blut vorhandenen Stoffe (Hormone) nur in einer ganz bestimmten, abgegrenzten Zeitspanne eine auslösende Kraft besitzen.

Die geschilderten Versuche sind natürlich nur ein allererster Anfang. Bis alle mit den Häutungs- und Verpuppungssinkreten zusammenhängenden Fragen gelöst sind, müssen noch eine Unzahl der sehr zeitraubenden Versuche ausgeführt werden. Wann treten die fraglichen Hormone auf? Wie greifen sie an? Und vor allem: wo werden sie gebildet? Zu dem letztgenannten Punkt können einige Vermutungen ausgesprochen werden. Zunächst ist an die Versonschen Beobachtungen an Oenocyten (s. oben S. 291) zu denken: Oenocytensekretion und Häutungen verlaufen in einem einigermassen einheitlichen Rhythmus. Doch ist mit dieser Feststellung natürlich eine ursächliche, auslösende Wirksamkeit des Oenocytensekretes noch lange nicht bewiesen. Fernerhin könnte man den oftmals beschriebenen Häutungsdrüsen neben ihren sekretorisch-mechanischen Funktionen auch inkretorische Fähigkeit zuschreiben. Schliesslich sei noch an die von Ostreykowna (1927) bei Raupen von *Plusia gamma* beschriebenen Bauchdrüsen erinnert. Diese von 7 Muskeln gehaltenen Organe liegen an der Grenze des Pro- und Mesothoracalsegmentes. Sie bestehen aus 3 Schichten, deren mittlere von grosskernigen hohen Drüsenzellen gebildet wird. Diese Drüsenkerne zeigen ein zu den Häutungen in zeitlicher Beziehung stehendes, wechselndes Aussehen. Der Zusammenhang der Bauchdrüsen mit den Bluträumen dürfte nachgewiesen sein. Endgültige Klarheit können natürlich auch hier nur zahlreiche eingehendste Versuche bringen.

Vielleicht gelingt es, später einmal alle diese Erscheinungen mit den von Brecher (1925) am Raupen- und Puppenblute durchgeführten physiko-chemischen Untersuchungen in Einklang zu bringen.

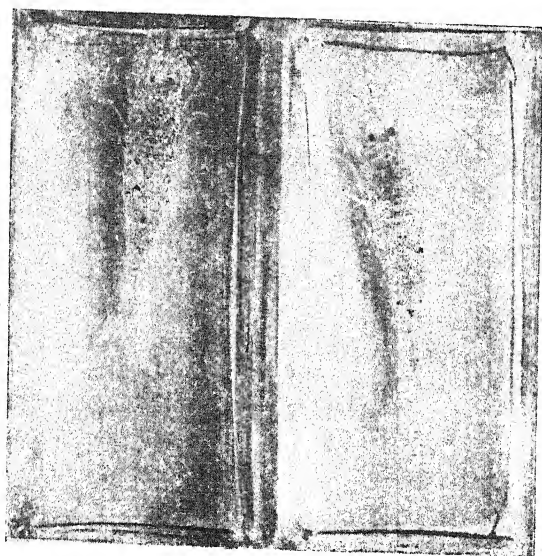
#### DIE FARBEWECHSELHORMONE.

*Garneelen.* Von allen Forschungszweigen, die bis heute zu der Frage nach Inkreten bei Wirbellosen führten, verdanken wir der Untersuchung des Farbwechsels—besonders des Farbwechsels der Garneelen (dekapode Krebse)—die aufschlussreichsten Ergebnisse. Dies mag zum grossen Teile seine Ursache darin haben, dass die Farbzellen (Chromatophoren), bzw. die Farbstoffe (Pigmente) jede natürlich oder experimentell bedingte—natürlich spezifische—Veränderung mit der Genauigkeit eines Manometers anzeigen. Dazu kommt, dass die Beantwortung jedes derartigen Reizes in verhältnismässig kurzer Zeit erfolgt, sodass alle störenden Einflüsse veränderlicher physiologischer Gegebenheiten ohne grosse Schwierigkeit ausgeschaltet werden können. Zudem sind die geeignetsten Versuchstiere (vor allem die Garneelen *Crangon*, *Leander*, *Palaemonetes*) leicht in der Gefangenschaft zu halten und durch grosse Widerstandskraft gegen operative Eingriffe ausgezeichnet.

Gewisse Garneelen, vor allem die Sandgarneele *Crangon vulgaris*, auf die im folgenden hauptsächlich Bezug genommen wird, zeigen ein erstaunliches Anpassungsvermögen an ihre Umgebung. Dies ist bedingt durch schwarzbraune, weisse, gelbe und rote Pigmente, die den von der Umgebung, vor allem dem Untergrund ausgehenden Reizen entsprechend, die zahlreichen verästelten Ausläufer (Chromorhizen) der Farbzellen mehr oder weniger zu erfüllen vermögen. Setzt man einen *Crangon* z.B. auf dunklen Untergrund, so beginnen alsbald die schwarzbraunen Pigmentkörnchen (Melanine) sämtliche Chromorhizen zu erfüllen (Expansion), während gleichzeitig das gelbe und weisse Pigment sich in der Mitte der syncytialen Chromatophoren zusammenballt (Kontraktion). Nach 30–60 Minuten ist dieser Vorgang im allgemeinen beendet. Verbringt man nun das völlig dunkel gewordene Versuchstier auf weissen Untergrund, so tritt in sämtlichen Farbzellen Kontraktion des Melanins und Expansion des weissen Farbstoffs ein. Ebenso bewirkt gelbe, bzw. rote Umgebung eine auffallend starke Ausdehnung der entsprechenden Pigmente, sodass die Versuchstiere eine gelbliche bzw. rötliche Färbung annehmen.

Die den Farbwechsel oder besser: die Farbanpassung bewirkenden Umgebungsreize werden nur vom Auge aufgenommen. Zweiseitig geblendete Tiere sind einer Untergrundsanpassung nicht mehr fähig. Die Frage, wie der vom Auge aufgenommene Reiz zu den Effektoren, eben den Farbzellen, geleitet wird, schien deshalb eine Untersuchung besonders zu lohnen, weil durch die Untersuchungen von Degner (1912) u.a. einwandfrei nachgewiesen war, dass die Chromatophoren nicht innerviert sind.

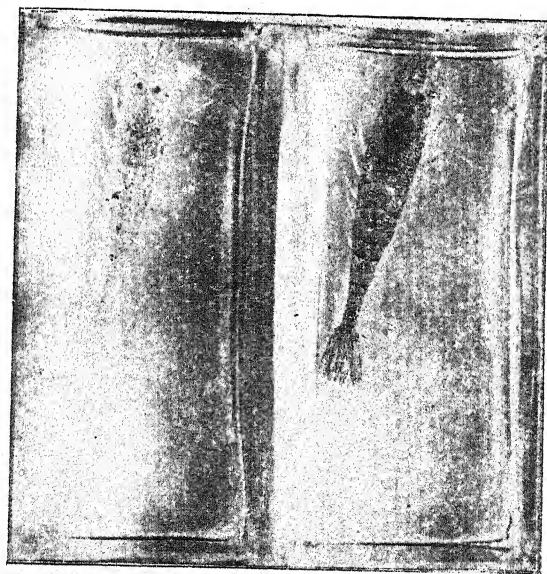
Zwei Arbeiten Kollers (1925 u. 1927) erbrachten nun den Nachweis, dass bei der Farbanpassung von *Crangon vulgaris* inkretorische Vorgänge am Werke sind. Entnimmt man einem dunkelangepassten Tier (Schwarztier) Blut und spritzt es einem Weisstier ein, so wird das Weisstier nach einigen Minuten deutlich dunkler,



A

B

Abb. 15. *Crangon vulgaris*. Zwei Weisstiere vor der Bluttransfusion. (Nach Koller.)



A

B

Abb. 16. Die gleichen Tiere wie Abb. 15. A (Kontrolltier) 10' nach der Injektion von Blut eines Weisstieres. B (Versuchstier) 10' nach der Injektion von Blut eines Dunkeltieres. (Nach Koller.)

obwohl es in weisser Umgebung belassen wird. Die durch Blutinjektion erreichte Dunkelfärbung hält 1–2 Stunden an, trotzdem das Tier unter optimalen Bedingungen zur Hellfärbung lebt. Kontrollversuche, Blutübertragung von Weisstier zu Weisstier, bewirken keinerlei Farbwechsellerscheinungen (vgl. Abb. 15 u. 16). In ganz ähnlicher Weise gelang es durch Blutübertragung von Gelbtieren auf Weisstiere Expansionen des gelben Pigmentes zu erlangen.

Trotz aller Bemühungen war es aber Koller nicht geglückt, Schwarztiere durch Einspritzung von Weisstierblut zur Hellfärbung zu bringen. Es war auf diesem Wege höchstens eine Verzögerung der Anpassungsgeschwindigkeit auf dunklen Untergründen zu erreichen gewesen. Der exakte Nachweis eines "Weisstoffes" gelang Perkins (1928), der an der Garneele *Palaemonetes* eine Reihe vorzüglicher Versuche ausführte. Zunächst bestätigte er durch die verschiedensten Eingriffe (Abklemmen von Blutgefässen usw.) die Haupttatsache, dass die Farbwechseleffektoren durch inkretorische Einflüsse zur Ausführung ihrer Bewegungen kommen.

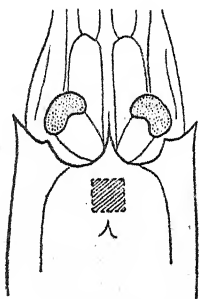


Abb. 17.

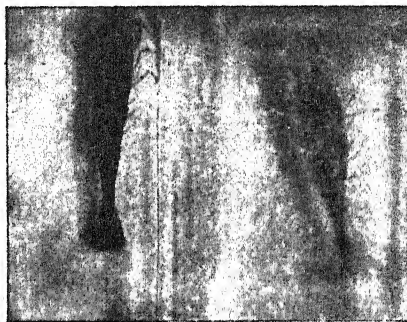


Abb. 18.

Abb. 17. *Crangon vulgaris*. Cephalothorax. Die schraffierte Stelle zeigt die ungefähre Lage des Schwarzorgans an. (Nach Koller.)

Abb. 18. *Crangon vulgaris*. Links Kontrolltier, rechts Versuchstier, dem das Schwarzorgan zerstört wurde. Beide Tiere nach mehrstündigem Aufenthalt auf schwarzem Grunde aufgenommen. (Nach Koller.)

Fernerhin vermochte er durch Einspritzung von Weisstier-Augenextrakten Schwarztiere zur Kontraktion der Melanine, also zur Hellfärbung, zu veranlassen. Dunkelfärbung durch Blutübertragung zu erreichen, gelang jedoch bei *Palaemonetes* nicht. Dieser Unterschied im Verhalten von *Crangon* und *Palaemonetes* mag darin begründet sein, dass bei *Crangon* überhaupt die Melaninexpansion, bei *Palaemonetes* jedoch—soviel ich aus Perkins' Arbeit entnehmen konnte—die Melaninkontraktion leichter hervorzurufen ist. Demnach würde bei *Crangon* beispielsweise der durch die Weisstierblut-Injektion gegebene Anreiz zur Melaninkontraktion durch die gegensinnige Wirkung des dunklen Untergrundes überwunden werden. (Es sei—in Parenthese—darauf hingewiesen, dass die natürlichen Aufenthaltsorte dieser beiden Garneelen sich bezüglich ihrer Untergrundfarben reziprok verhalten zu den vorherrschenden Farbwechselneigungen ihrer beiden Bewohner: *Crangon*

lebt auf hellem Sand und wird leichter dunkler, *Palaemonetes* hingegen lebt im dunklen Seegras und wird leichter hell.)

Nach den geschilderten Versuchen müssten also—wenigstens mit der allergrössten Wahrscheinlichkeit—inkretorische Organe angenommen werden, die auf den von den Sehelementen aufgenommenen und wahrscheinlich über das Zentralnervensystem auf nervösem Wege zugeleiteten Reiz hin spezifische Stoffe (Hormone) absondern. Diese gelangen auf dem Blutwege zu den Chromatophoren und lösen dort die Pigmentbewegungen aus.

Wo liegen nun die in Frage stehenden Organe? Die Versuche von Perkins zeigen, dass ein die Melaninkontraktion bewirkendes Organ ("Weissorgan") in den Augen, bzw. den Augenstielen liegt. Dieser Befund wurde von Koller (1928) für mehrere Garneelenarten bestätigt. Weiterhin konnte aber Koller auch für das "Schwarzorgan" die Lage in der vordersten, medianen und dorsalen Gegend des Cephalothorax, kurz "Rostralgegend" genannt, ermitteln. Dies gelang sowohl durch Injektion von Extrakten aus dieser eng umgrenzten Körperstelle (s. Abb. 17), als auch durch ihre Verfütterung. Am klarsten liess sich die Lage des "Schwarzorgans" aber dadurch zeigen, dass nach Zerstörung (Ausbrennung) der Rostralgegend die Versuchstiere die Fähigkeit des Dunkelwerdens völlig verloren haben, obwohl die Augen und das Nervensystem unverletzt geblieben waren (s. Abb. 18). Vermutlich gehört das Schwarzorgan zu den bis jetzt als Lymph- oder Blutdrüsen beschriebenen Gebilden.

Dass von Perkins und Koller bei ihren Versuchen die jeweils notwendigen Kontrollversuche durchgeführt wurden, ist selbstverständlich. Die Wirkungsweise der verschiedenen Injektionsstoffe seien in der beigegebenen Tabelle Nr. 8 zusammengefasst.

Tabelle 8.

Spender		Farbänderung des Empfängers nach der Injektion	
		Weisstier	Schwarztier
Injektionsstoffe von Weisstieren	Blut	—	—
	Augenextrakt	—	Starke Aufhellung
	Extrakt aus der Rostralgegend	Verdunklung (wenigstens bei geblendeten Tieren)	—
Injektionsstoffe von Schwarztieren	Extrakt aus Abdominalsegmenten	—	—
	Blut	Verdunklung	—
	Augenextrakt	—	Schwache Aufhellung (PERKINS)
	Extrakt aus der Rostralgegend	Verdunklung	—
	Extrakt aus Abdominalsegmenten	—	—

Dass die Farbwechselstoffe tatsächlich hormonaler Natur sind, wird durch verschiedene Versuche Kollers sehr wahrscheinlich gemacht:

(a) Die Fütterungsversuche zeigen, dass die Farbwechselstoffe auch nach Einverleibung per os, also auch nach Durchgang durch den Verdauungstraktus, ihre spezifische Wirksamkeit behalten.



(b) Die Farbwechselstoffe sind, wie wir das ja auch von Wirbeltierhormonen wissen, weder art- noch gattungseigen. Wählt man nämlich Spender und Empfänger aus 2 verschiedenen Gattungen (z. B. *Leander serratus*—*Processa canaliculata* oder *Crangon vulgaris*—*Leander squilla*), so haben trotzdem die Injektionsstoffe die gleiche Wirkung, als wenn es sich um Tiere derselben Art handelte.

(c) Aus einer noch nicht veröffentlichten Versuchsserie (1928–9) sei mitgeteilt, dass sowohl die Augen- als auch die Rostralgegendextrakte *selbst nach längerem Kochen noch ihre volle Wirksamkeit* behalten.

Damit dürfte mit weitgehender Sicherheit dargetan sein, dass es sich bei den Farbwechselstoffen keinesfalls um Fermente handelt. Ihre den Wirbeltierhormonen in vielen Punkten gleichlaufenden Eigenschaften lassen sie als echte Inkrete erscheinen.

*Dixippus*. Aufbauend auf den Arbeiten von W. Schleip (1910, 1915, 1921) hat neuerdings Giersberg (1928) den Farbwechsel der Stabheuschrecke *Dixippus morosus* sehr gründlich untersucht. Auch Giersberg kommt bei seinen Versuchen zu dem Schluss, dass Hormone in den Farbwechsel-Reflexbogen eingeschaltet

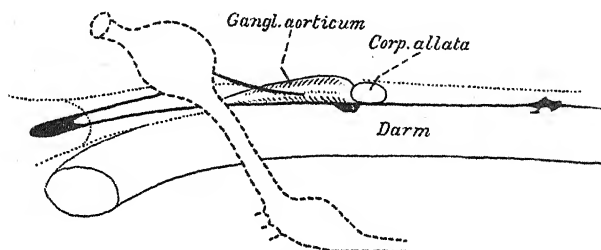


Abb. 19. *Dixippus morosus*. Gehirn, sympathisches Nervensystem und Corpora allata. (Nach Giersberg.)

sind. Zusammenfassend schreibt dieser Autor über die Wirkung hoher Luftfeuchtigkeit, die in kurzer Zeit Verdunklung der Stabheuschrecken bedingt: "Die Feuchtigkeitsreize können von allen Bezirken des Körpers aufgenommen werden, werden durch das Bauchmark ins Gehirn geleitet und dann über das Ganglion frontale ins sympathische Nervensystem übertragen. Das sympathische System bewirkt, offenbar durch Ausscheidung bestimmter Stoffe, eine Veränderung des Blutes. Die Veränderung des Blutes aber bewirkt die Veränderung der Farbzellen, die mit dem betreffenden Blut in Berührung kommen. Der Weg der physiologischen Farbbeeinflussung bei *Dixippus* ist also der 'hormonale'."—Ob tatsächlich die sympathischen Nerven selbst die betreffenden Stoffe ausscheiden oder ob die Bildungsstelle der Hormone die schon durch Heymons (1899) beschriebenen Corpora allata (vgl. Abb. 19) sind—die übrigens Giersberg selbst in diesem Zusammenhang nennt—möge einstweilen dahingestellt sein. Allerdings zeigen die in Frage kommenden Nerven engste Beziehungen zu den Blutgefäßwandungen, andererseits sind an den Corpora allata mitunter Sekretionserscheinungen zu beobachten. Die von Giersberg angekündigten weiteren Untersuchungen werden sicherlich eine Klärung der angeführten Probleme bringen.

*Cephalopoden.* Die Pigmentbewegung in den Farbzellen der Cephalopoden wird bekanntlich durch innervierte glatte Muskeln bewirkt. Trotzdem ist die Beteiligung von Inkreten nicht ausgeschlossen (vgl. Sereni, 1928).

#### ANHANG: WIRKUNG VON WIRBELTIERHORMONEN AUF WIRBELLOSE.

Bei dem geringen Mass von Tatsachen, das uns bis heute über die Hormone der Wirbellosen zur Verfügung steht, mag es wohl angebracht sein, kurz und anhangsweise noch die Untersuchungen zu erwähnen, die sich mit der Einwirkung von Wirbeltierhormonen auf wirbellose Tiere beschäftigen. Leider wurden derartige Untersuchungen oft unter pharmakologischen nicht aber unter vergleichend-physiologischen Gesichtspunkten angestellt. Natürlich darf aus diesen Versuchen—soweit sie überhaupt von bemerklichen Einwirkungen berichten—niemals der

Tabelle 9.

Autor	Verfüttertes Hormonalorgan	Versuchstiere	Untersuchte Lebenserscheinungen, etc.	Wirkung	Bemerkungen
Nowikoff (1908)	Hypophyse	<i>Paramecium</i>	Vermehrung	Stark fördernd	Konz. $\frac{1}{3}$ -1 %
Hanko (1912)	„	<i>Asellus</i>	Häutung, Regeneration, Wachstum	„	—
Abderhalden (1919)	„	<i>Deilephila euphorbiae</i>	Grösse des Falters	Fördernd	—
Nowikoff	Thyreoidea	<i>Paramecium</i>	Vermehrung	Stark fördernd	Kontrollversuche?
Abderhalden	„	<i>D. euphorbiae</i>	Grösse des Falters	Hemmend	—
Romeis und Dobkiewicz	„	<i>Calliphora vomitoria</i>	Wachstum u. Entwicklung	Anfangs etwas hemmend, dann ohne Wirkung	—
Resničenko (1927)	„	<i>Drosophila</i>	„	Ohne Wirkung	Auch nicht b. K'-zusatz
Dobkiewicz (1928)	„	„	Entwicklung u. Grösse	„	—
Weiss (1928)	„	<i>Ciona</i>	Entwicklungsgeschwindigkeit	Deutlich beschleunigend	Viele Kontrollversuche
Nowikoff	Nebenniere	<i>Paramecium</i>	Vermehrung	Schädlich	—
Abderhalden	„	<i>D. euphorbiae</i>	Grösse des Falters	Stark hemmend u. verbildend	—
Bauer (1926)	„	<i>Paramecium</i> u. <i>Leucocyten</i>	Zustand des Plasmas	Zäherwerden, Verhinderung v. Strömungen	—
Sereni (1928)	„	Cephalopoden	Chromatophoren, Muskel-tätigkeit	Expansion der Chr. Pupillenerweiterung. Steigerung d. motorischen Tätigkeit	N.B. Verabreichung durch Injektionen
van Herwerden (1923)	Nebennierenrinde	<i>Daphnia</i> und <i>Limnaea</i>	Wachstum, Fortpflanzung, Widerstandskraft	In kleinen Dosen sehr stark fördernd	Bes. wirksam Nebenniere v. schwangeren Tieren

Schluss gezogen werden, dass die betreffenden Hormone in dem Versuchstier unter natürlichen Bedingungen vorkämen. Die Ergebnisse der wichtigsten Arbeiten auf diesem Gebiet seien, der besseren Uebersicht wegen, in Tab. 9 wiedergegeben.

Eindeutig scheint demnach nur die wachstumsfördernde Wirkung des Hypophysenhormons nachgewiesen zu sein. Ob die Unterschiede in der Wirkung des Schilddrüsenhormons auf der Verschiedenheit der Versuchstiere oder der Versuchsanordnung beruhen, ist nicht festzustellen. Besondere Beachtung verdienen die Versuche von van Herwerden an *Daphnia pulex* und *Limnaea ovata*, deren Ergebnisse wohl ebensowenig angezweifelt werden können, wie die Untersuchung von Weiss (1928) an *Ciona*. Die letztgenannte Arbeit ist von besonderem vergleichend-physiologischen Interesse, lassen ja doch Verwandtschaftsbeziehungen der Ascidien zu den Wirbeltieren (vgl. Maurer, 1906) auch auf eine ähnliche Wirkungsweise ihrer Inkrete schliessen.

#### SUMMARY.

1. The presence of active *sexual hormones* can be assumed with a reasonable degree of certainty in cases where parasitic or experimental castration brings about specific changes in secondary sexual characters. One of the principal instances of this is the *parasitic castration* of decapod crustacea (*Carcinus*, *Inachus*, *Pagurus*) by Rhizocephala (Giard, Smith, Potts, Nilsson-Cantell, van Oordt), the result of which is, in male crabs at least, a definite approach to the female facies. It seems possible that the seat of formation of the hormones is not in immediate connection with the gonads themselves (Courrier). Similarly "stylopisation" in the hymenopteran *Andrena* causes alterations in colour of the clypeus, differentiation of the tibia, etc., in both sexes, resulting in an unmistakable approach to the opposite sex (Pérez, Wheeler, etc.). Earthworms (*Lumbricus herculeus*), in which the testes are destroyed by parasites, lack the clitellum, a secondary sexual organ. This natural phenomenon has been confirmed experimentally by Harms.

The results of *experimental castration* indicate, as far as the present state of our knowledge goes, that there is a fundamental difference between crustacea and insects. It has been definitely proved by the castration of *Asellus aquaticus* with radium that in this animal the development of the brood pouch is dependent upon the presence of functional ovaries (Haemmerli-Bovari). The numerous gonad extirpations and transplantations performed on caterpillars by Meisenheimer, Oudemanns, Kopeć, Prell, and others, do not permit of any certain assumption that hormones exist in these animals. The same is true for experiments with *Grillus campestris* (Regen). It is nevertheless the opinion of some workers (Harms, von Buddenbrock) that the gonads may be spacially separated from the endocrine organs, as in Courrier's experiments mentioned above, and that consequently castration experiments cannot settle the matter.

2. In *Physcosoma* Harms has demonstrated histologically and physiologically the existence of an endocrine gland (*internephridial organ*) the secretion of which is essential to the life of the animal.

3. In *Cephalopods* the morphological characters of the branchial and pericardial

glands suggest endocrine action. The fact that tyramine, a product of the salivary glands, has also been found in the blood (Henze, Sereni) is of particular significance.

4. The *oenocytes of larval and adult insects*, which have been studied by numerous workers, are unicellular endocrine glands. The secretory process originates in the nucleus of the oenocyte. Probably the oenocyte endocrines are of importance in metabolism, and perhaps also in developmental processes such as ecdysis.

5. Koller has shown by blood transfusion that internal secretions are probably concerned in *ecdysis and pupation of caterpillars*. The place of formation of the hormones concerned is still unknown.

6. Koller and Perkins have shown experimentally that the expansion and contraction of melanin in the *chromatophores of shrimps and prawns* is due to substances which are secreted into the blood of the animals in response to light stimuli. The seats of formation of the two endocrines are situated respectively in the eyes and in the rostral region of the animals. Koller showed that these substances do not lose their efficacy either by passing through the wall of the alimentary canal or by boiling. This, together with the fact that they are neither specific for species nor for genera, confirms the endocrine nature of the substances in question.

The investigations of Giersberg on the *colour changes of Dixippus* lead to the assumption of hormone action here also. The sympathetic nerves and the corpora allata are regarded as the places of formation of the substances concerned in the colour change.

7. The *feeding of invertebrate animals with vertebrate hormones* has not yet led to definite and unambiguous conclusions. Up to the present the experiments are for the most part merely of pharmacological importance.

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## THE CHEMICAL CHANGES DURING THE METAMORPHOSIS OF INSECTS

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(Received May 13, 1929.)

(With Nine Text-figures.)

THERE is no need to emphasise the interest which biologists have always taken in insect metamorphosis. "Those strange and mystical Transformations," wrote Sir Thomas Browne, "that I have observed in silkwormes, turned my Philosophy into Divinity, for there is in these works of Nature, which seem to puzzle reason, something Divine, and hath more in it than the eye of a common spectator doth discover." And on August 30th, 1666, at the Royal Society "Sir Robert Moray mentioned that the King had been discoursing of Ant's egges, and inquiring how they came to that bignesse which sometimes exceeded that of the insect itself." During the two following centuries the advances made were mainly morphological, but there were not wanting, even in the early stages, scattered observations of a chemical character. In 1807 Spallanzani<sup>(32)</sup> noticed that the larvae and butterflies of a given species respired twice or thrice as intensely as the chrysalides, and this was confirmed by Newport<sup>(27)</sup> who in 1836, with much more accurate technique, measured the respiration of *Sphinx* pupae. He obtained U-shaped curves similar to those which will later be discussed in this review, as also did Regnault and Reiset<sup>(28)</sup>, working on the metamorphosing May beetle thirteen years later.

In order to obtain as synthetic a view as possible of the relationship of the various chemical changes to each other and to the accompanying morphological changes, all the available data for the silkworm, *Bombyx mori*, are first summarised, as this insect has been used by perhaps more workers than any other.

The course in time taken by the life-history of the silkworm varies with the external conditions, but as an average picture the following may be used. On the thirty-second day of larval life the worm begins to spin the cocoon and this spinning takes about three days. Two or three days after the cocoon is finished the fifth moult occurs inside it (four having taken place during larval life). The period from the beginning of spinning to the coming out of the fly occupies fifteen to twenty-one days.

The larval period is a time of immense growth; then, from the beginning of spinning there is a fall in weight, at first rapid, afterwards more gradual. When the insect is ready to make its cocoon it leaves off feeding, evacuates all the solid matter contained in its digestive tube and finds a suitable place where it may settle down and attach itself for the spinning. During the latter process the silkworm is continually executing a figure-of-eight movement with the anterior part of the body.

When the cocoon is finished, the insect places itself inside and remains motionless until the moult takes place; this requires some sudden contractions of the body to split and remove the skin and to push it to the hind part of the chrysalis. After this the pupa remains motionless until the imago is well developed. The figures given by Vaney and Maignon<sup>(37)</sup> for the wet weight on each day of pupal development, both for the pupa and cocoon and for the naked pupa, are plotted in Fig. 1. It is seen that the greatest loss of weight occurs during the first four days, *i.e.* during the time of the spinning. By the end of the pupal period the naked pupa weighs only about half as much as the ripe larva; the fly which emerges, leaving behind the pupal skins, is only one-quarter to one-third the weight of the ripe cocoon. During the larval period, according to Kellner, Sako and Sawano<sup>(23)</sup>, the insect becomes slightly wetter, the percentage of dry weight to wet weight falling from 23.6 to 19.6. At the ninth day, after the beginning of spinning, the percentage of dry weight has risen to 29.1 and in the fly it is 28.2. The greater part of the loss of weight (about 60 gm. for 100 individuals) during pupation must be due to simple loss of water. Thus Kellner, Sako and Sawano found 139.5 gm. of water in 100 worms and 37.4 of dry solid, while in 100 cocoons and their pupae there were only 83 gm. of water and 33.9 gm. of dry solid.

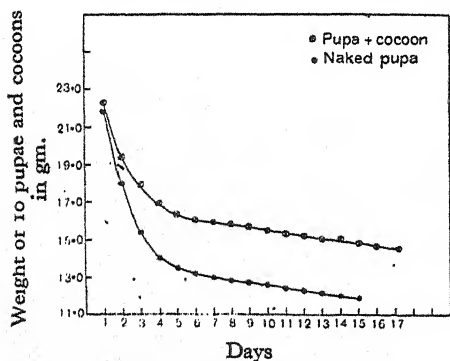


Fig. 1. Vaney and Maignon.  
Weight loss in *Bombyx*

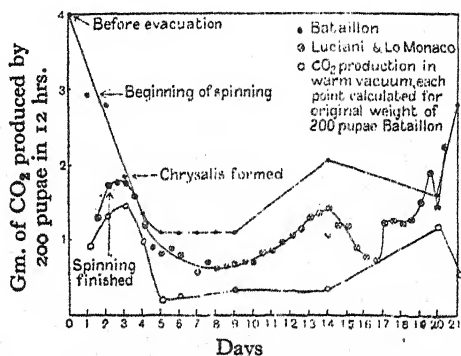


Fig. 2. CO<sub>2</sub>-production in *Bombyx*.

The next thing to notice about the developing pupa is the change which takes place in the CO<sub>2</sub>-production and therefore presumably in the intensity of respiration. This does not seem to have been studied for the silkworm since 1893, but the results then obtained by Bataillon<sup>(4)</sup> and by Luciani and Lo Monaco<sup>(26)</sup> agree in general outline with those obtained more recently for other pupae. From Fig. 2 it is clear that after the beginning of the chrysalis stage (*i.e.* after the fifth moult) there is quite good correspondence between the two sets of results. For two or three days there is a fall in the carbon dioxide output, followed by a more or less steady low level persisting until the sixth or seventh day after the beginning of the chrysalis stage. Then there is a steady rise until the eleventh day, followed by a drop (shorter in time and greater in amount in Luciani and Lo Monaco's experiment), which is again succeeded by a sudden rise as the moths begin to

emerge. According to Bataillon, carbon dioxide output is at a maximum on the day before spinning begins, and then falls steadily—but in Bataillon's account the interval between the beginning of spinning and the fifth moult seems extraordinarily short, only two days. According to Luciani and Lo Monaco the carbon dioxide output is increasing during the period immediately succeeding spinning and prior to the shedding of the skin. This pre-chrysalis period, interesting because of the reactions involved in synthesising the silk, and also because of the spinning movements, needs further investigation.

Bataillon also investigated the amount of carbon dioxide which could be extracted from the pupae warmed in a vacuum at different stages of development. His results show that, using the same weight of pupae at each stage, a curve with a peak at the time of formation of the chrysalis, followed by a drop to a steady low value, which later rises and falls again, is obtained. These variations may be due to variations in the alkaline reserve caused, *e.g.* by the production of non-volatile acids.

Claude Bernard (6) was probably the first to observe the enormous accumulation of glycogen during larval life. "*La larve d'Asticot*," he remarked, "*c'est un véritable sac de glycogène*," but he could find no trace of glucose in the larva, although in the chrysalis and the adult he observed both glycogen and glucose. In 1892 Bataillon and Couvreur (5) attacked the question, and more extensive experiments were published by Bataillon (4) in the following year. Using the same material as for the carbon dioxide experiments, Bataillon found a maximum glycogen content of 0.9 gm. in 100 individuals in the chrysalids one day old (see Fig. 3); after this point there was a rapid fall, and towards the end of pupation the quantities present were too small to estimate. During the time of spinning and moulting the glycogen is piled up very rapidly, the quantity being more than doubled during the period. During this time too, according to Bataillon, glucose first appears, and towards the end of the spinning rises rapidly, then more slowly until the fifteenth day after the appearance of the chrysalis, when it falls quickly. The general character of Bataillon's curve for glycogen is well borne out by that of Vaney and Maignon (37) in 1905; the latter workers found much larger amounts of glycogen, but this is probably due to differences in the diet of the larvae. The maximum content (2.7 gm. per 100 individuals) was found just at the time of appearance of the chrysalis. The figures for glucose are much smaller and more variable than those of Bataillon, never being greater than 0.18 gm. for 100 individuals, and never approaching the glycogen in amount. The time of the first appearance of glucose they also found to be very variable.

In 1909 Kotake and Sera (24) could find no rise in the glycogen content after the stage of the ripe worm. This may be because they did not do their estimations at the right times—the second estimation in their tables is always the first or second day "*nach dem Einspinnen*." If this means after the end of spinning the maximum may have been missed; if it means after the beginning of spinning (as has been assumed in Fig. 3) then it is possible that the exact date of the maximum varies, sometimes being at the ripe worm and sometimes at the chrysalis stage. Kotake and Sera's figures have been recalculated for 100 pupae, using Vaney and Maignon's



loss of weight curve. Inouye<sup>(19)</sup> in 1912 found a total carbohydrate content for 100 silkworms of 2.11 gm., and for 100 pupae at the ninth day after the end of spinning, 0.88 gm. Similarly, in 1884, Kellner, Sako and Sawano<sup>(23)</sup> found for the "nitrogen-free extractives" in 100 silkworms ready to spin a value of 5.88 gm., and in 100 pupae about six days after the beginning 1.41 gm.

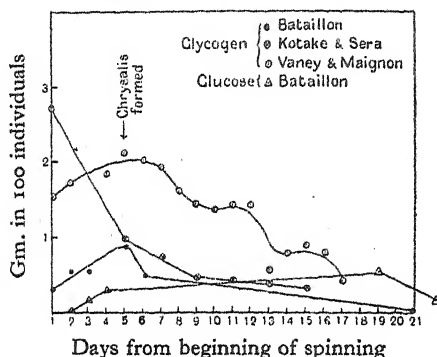


Fig. 3. Carbohydrates in *Bombyx*.

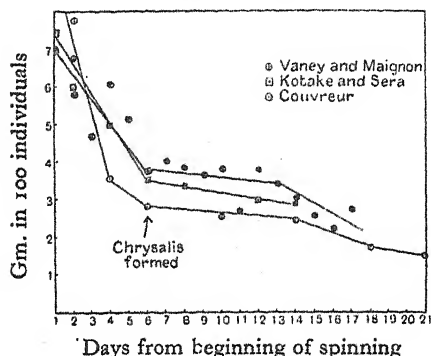


Fig. 4. Fat in *Bombyx*.

The same workers who studied the carbohydrate changes have also examined the fat content of *Bombyx mori* at different stages of development (Couvreur<sup>(11a)</sup>, Vaney and Maignon<sup>(37)</sup>, Kotake and Sera<sup>(24)</sup>) and their results are in good agreement. A rapid fall in the first four or five days is followed by a period of very slight decrease, and finally again by a period of more rapid disappearance; about 5 gm. of fat are lost by 100 pupae (Fig. 4). Kellner, Sako and Sawano<sup>(23)</sup> also give figures for the fatty acid content of 1000 worms nearly ready to spin, and 1000 pupae "some days after pupation." The former value is 71.2 gm. and the latter 61.2. This loss of fat is exceedingly small compared with that found by all other workers. Couvreur saw in the fat disappearing in the pre-chrysalis period the origin of the glycogen which he and Bataillon<sup>(5)</sup> (and also Vaney and Maignon at a later date) found rising to a maximum during this time. Kotake and Sera, as we have seen, did not find this glycogen maximum during spinning, but found that both glycogen and fat disappeared from the time of the beginning of the cocoon. They therefore denied any conversion of fat into glycogen. Weinland<sup>(42)</sup> discussed the question in 1907 in comparing the events in *Bombyx* with those in *Calliphora*, on which insect his own work was done. He considered that the coincidence in time of the disappearance of fat and the formation of glycogen could not, in the absence of any other evidence, be looked upon as proof of the conversion of one into the other. In a *Brei* of the pupae of *Calliphora*, where an increase in carbohydrate was also observed, the evidence seemed to be against its formation from fat, and Weinland suggested protein as its source. He thought that the fat disappearing at the beginning might be used to provide the necessary energy for the work involved in spinning.

According to Kellner, Sako and Sawano, three-quarters of the non-nitrogenous extractives are destroyed during formation of the pupa, the fat being conserved

and only about one-seventh of it being used. Then they say that in the last part of the pupal stage, the last quarter of the nitrogen-free extractives is used and the fat is also consumed, less than two-thirds of it remaining at the end of pupation. This description does not agree in detail with that of all the other workers.

With regard to the metabolism of nitrogenous substances, the chief change from a quantitative point of view is the formation of the silk. This is secreted from the silk glands in a semi-liquid form, and according to Kellner, Sako and Sawano<sup>(23)</sup> contains 87.5 per cent. of dry solid, of which 98.8 per cent. is protein and 1.18 per cent. ash. About half the protein of the ripe worm is used to make the silk, and appears in the cocoon still as protein though of a different composition. The newly-hatched moth contains less protein than the newly-formed pupa (with the cocoon removed) and the protein disappearing during this period is probably combusted, most of its nitrogen appearing as uric acid, which is excreted by the moth at birth. Some of the nitrogen probably also goes to form the chitin of the adult. The following figures, taken from Kellner, Sako and Sawano and from Inouye<sup>(19)</sup>, show some of these relationships.

	Gm. per 100 individuals	
	Total nitrogen	Crude protein
Kellner, Sako and Sawano:		
Mature silkworm ... ..	4.26	25.8
Naked pupa ... ..	1.99	12.1
Cocoon ... ..	2.20	—
Inouye:		
Mature silkworm ... ..	4.79	—
Naked pupa ... ..	1.76	—
Cocoon ... ..	2.96	—
Finished moth ... ..	1.39	—
Larval skin cast on pupation (chitin) ... ..	0.07	—
Pupal skin cast on appearance of moth (chitin) ... ..	0.14	—
Urates excreted by moth ... ..	0.26	—

Kellner, Sako and Sawano remark that the reddish excreta of the newly-emerged moths give a very marked qualitative test for uric acid. From 1000 moths 60.5 gm. dry solid were excreted, and as, according to Karmrodt<sup>(21)</sup>, it contains 17 per cent. of nitrogen, about 10 gm. of nitrogen were excreted, which is more than Inouye found. If the nitrogen is assumed to be present wholly in the form of uric acid, this would be equivalent to about 30 gm. The table shows incidentally an interesting fact which had previously been shown to be true by Kellner, Sako and Sawano directly, namely, that no nitrogen is lost in gaseous form during metamorphosis.

Next, it is interesting to enquire into the distribution of the nitrogen contained in the organism during the different stages, during metamorphosis. This is important from two points of view. Firstly, during the great changes in structure which go on in the transformation of the organs of the larva into the organs of the moth, it seems essential to suppose that the protein making up the former must be brought into a soluble form and possibly even broken down to amino-acids

before it can be used for the building-up of the organs of the imago. If this is so, we might expect to find the morphological upheaval reflected in a raised proportion of peptone, polypeptide, and amino-acid nitrogen to protein nitrogen. The removal of the larval organs and the shaping of the new ones probably go on concurrently, so that no great changes in non-protein nitrogen need be expected, but it would be surprising if there was no such reflection of the morphological changes. Secondly, if it is true that some protein is burned as a source of energy during pupation, and if no nitrogen is lost during the same period, then the end-products of protein breakdown will accumulate and so raise the non-protein nitrogen fraction. Unfortunately, although we know the nitrogen excreted by the newly-emerged moth, we do not know whether this excrement comprises the whole of the nitrogenous waste or whether any uric acid is left behind with the pupal case and cocoon; also there have been no estimations of nitrogenous products of combustion at the ninth day after the end of spinning or at the ninth day after the beginning of spinning, which were the times chosen by Inouye and by Kellner and his collaborators respectively for the investigation of the nitrogen distribution. Moreover, it is still unknown whether uric acid formed during larval life may remain in the body of the pupae. Wigglesworth<sup>(43)</sup>, working on *Pieris brassicae* and *Vanessa urticae*, found that the same quantity of uric acid was contained in the resting pupa as in the pupa just before emergence and stated that there was no evidence for the formation of uric acid during pupal life in these types.

Kellner gives the following table:

Gm. in 100 gm. dry weight	Mature silkworm	Cocoon	Naked pupa	Moth
Crude protein ... ..	59.16	98.8	55.8	56.6
Chitin ... ..	4.77	—	3.89	7.38
Total nitrogen ... ..	9.75	17.97	9.16	9.49
Protein and peptone nitrogen ... ..	8.11	—	5.68	8.18
Chitin nitrogen ... ..	0.29	—	0.23	0.44
Nitrogen not precipitable by phosphotungstic acid ... ..	1.35	—	3.25	0.87
Nitrogen not precipitable by phosphotungstic acid in % of total nitrogen ...	13.8	—	35.5	9.2

Perhaps the most important facts which emerge from the table are these. Firstly, while the percentage of total nitrogen in the dry substance is very much the same for the mature worm, pupa, and moth, the percentage of protein and peptone nitrogen is lower in the pupa than in either of the other stages; secondly, the percentage of nitrogen (in the dry substance) not precipitable by phosphotungstic acid is highest during the pupa stage; thirdly, the relation of nitrogen not precipitable by phosphotungstic acid to total nitrogen is much higher at the pupal than at the other stages. At first sight these figures seem to show a fall in relative amount of protein nitrogen at the pupal stage, which, as it is followed by a marked rise in the moth, might be regarded as associated with histolytic changes. It must be remembered, however, that the figures for the moth exclude the excreta, and if we allow for these the picture is considerably modified. If we take Inouye's

figures—as being perhaps more accurate than those obtained by combining Kellner's and Karmrodt's work—for the nitrogen and those of Kellner, Kakizaki, Matsuoka and Yoshii<sup>(22)</sup> for the dry weight of moths, we obtain the following results (assuming that the uric acid has all been formed during pupal life):

100 moths or 14.6 gm. dry weight excrete 0.26 gm. nitrogen;

therefore 100 gm. dry weight excrete 1.8 gm. nitrogen.

This 1.8 gm. we may suppose to be contained in about 10 gm. of excreta, so the table now reads:

Gm. in 100 gm. dry weight	Mature silkworm	Pupa	Moth
Total nitrogen ... ..	9.75	9.16	10.3
Protein and peptone nitrogen ... ..	8.11	5.68	7.4
Nitrogen not precipitable with phosphotungstic acid ... ..	1.35	3.25	2.42
Nitrogen not precipitable with phosphotungstic acid in % of total nitrogen ...	13.8	35.5	23.5

We see now that, allowing for the excreta, the pupal stage still presents a minimum percentage of protein nitrogen and a corresponding maximum percentage of non-protein nitrogen, but it must be remembered that if we had used the much higher figures calculated from the work of Karmrodt and of Kellner, Sako and Sawano, or if, on the other hand, only part of the uric acid has been formed during pupation, the results would have been very different.

Inouye also studied the distribution of nitrogen, and his results are summarised in the following table:

Gm. % dry weight	Mature silkworm	Pupa	Moth
A Total nitrogen ... ..	11.23	8.87	10.49
B Protein nitrogen ... ..	8.64	6.03	7.65
C Nitrogen of the bases ( <i>i.e.</i> in the phosphotungstic precipitate after removal of proteins) ... ..	1.11	0.85	0.96
D Nitrogen not precipitable by phosphotungstic acid	1.49	1.89	1.84

This table may be reconstructed, adding on the 2 gm. of non-protein nitrogen excreted by 100 gm. dry weight of moth, and thus we obtain:

A	11.23	8.87	9.53
B	8.64	6.03	6.98
C	1.11	0.85	0.85
D	1.49	1.89	3.30
D in % of A	13.3	21.3	36.7

These results agree with those of Kellner and his collaborators in showing a minimum of protein nitrogen per cent. dry weight at the pupal stage, but, unlike theirs, they show a maximum of non-protein nitrogen at the moth stage. Even in the moth alone, not counting excreta, Inouye found almost as high a percentage of nitrogen not precipitable by phosphotungstic acid as in the pupa. The difference between the results of Inouye and those of Kellner and his collaborators may

perhaps in part be explained by the fact that Inouye used pupae at a stage of development some three to five days later than those used by Kellner. His figures for nitrogen not precipitable by phosphotungstic acid are higher than Kellner's for the moth, and lower for the pupa. Inouye also estimated the water-soluble nitrogen, but as he does not mention the pH of his extracts it is impossible to say what nitrogenous compounds he had in his solution. The results were as follows:

Gm. % dry weight	Mature silkworm	Pupa	Moth
Water-soluble nitrogen ... ..	3.12	2.74	3.11
Water-soluble nitrogen in % of total nitrogen	27.7	30.9	32.6

If the excreta were counted, the figures in the last column would be 5.11 and 53.6 respectively.

It is clear that much more work is needed to follow the accumulation of protein end-products during metamorphosis, and also to distinguish at the different stages the nitrogenous compounds playing an intermediary part between the breakdown of the old tissues and the formation of the new ones.

Fischer and Skita<sup>(13)</sup> in 1901 showed that the silk fibrin, which constitutes about 70 per cent. of the raw silk, contains high percentages of glycine (36.0), alanine (21.0) and tyrosine (10.5) as well as a little leucine, phenyl alanine, serine, aspartic acid, and proline. Abderhalden and Behrend<sup>(1)</sup> later found very similar results. Abderhalden and Dean<sup>(2)</sup> hydrolysed ripe silkworms, and estimated by the ester method the acids formed. Similarly Abderhalden and Weichardt<sup>(3)</sup> analysed the protein of the newly-emerged moths. As shown in the following table, there is a great drop in the percentage of glycine, alanine, and tyrosine, and Abderhalden was of the opinion that the silkworm ready to spin contains enough of these amino-acids to provide the amount contained in the cocoon. It is therefore unnecessary to suppose any synthesis of them during the spinning process.

	Silkworm %	Moth %
Glycine ...	10.2	3.5
Alanine ...	8.7	3.2
Valine ...	1.7	1.7
Leucine ...	4.8	8.5
Aspartic acid	1.6	2.7
Glutamic acid	3.5	5.7
Phenylalanine	2.4	2.7
Tyrosine ...	4.3	1.6
Proline ...	1.5	4.0

Inouye also carried out hydrolysis experiments at different stages, and gives the following table for the hydrolysate:

Gm. per 100 gm. nitrogen	Mature silkworm	Cocoon	Pupa	Moth
Nitrogen in phosphotungstate precipitate	21.37	6.23	24.09	24.62
Nitrogen in filtrate ... ..	70.54	89.97	64.96	61.94

This illustrates very well the utilisation of monoamino acids for the formation of the cocoon.

Finally we must consider the question of the chitin content, and here there is some confusion in the literature. Kellner, Sako and Sawano give, for the dry weight of 1000 larval skins moulted inside the cocoon, the value of 0.8 gm., of which 90 per cent. or 0.72 gm. was organic substance (we will assume all chitin) and the rest ash. Inouye in one case found that the larval skins from 100 pupating silkworms contained 0.07 gm. nitrogen; if we assume that this nitrogen is all in the form of chitin, then since chitin contains about 7 per cent. of nitrogen, we obtain by calculation 1 gm. of chitin. This is a little more than ten times the value given by Kellner and his associates, and, as Inouye's value agrees much better with the chitin differences (see below) given in Kellner's tables, it seems probable that the 1000 larval skins mentioned by Kellner is a misprint for 100.

The following table for chitin content is given by Kellner, Sako and Sawano:

	Chitin, gm. per 1000 individuals
Mature silkworm	20.82
Pupa ... ..	8.47
Empty cocoon ...	—
Moth ... ..	10.47

Therefore loss, *i.e.* worm to pupa, = 12.35 gm. and gain in passing from pupa to moth = 2.0 gm.

This 12.35 gm. corresponds well enough with the 10 gm. found by Inouye for 1000 larval skins. Inouye also found about the same amount of nitrogen in the pupal skins cast on emergence of the moth; Kellner, however, does not seem to have considered this in computing the chitin formed during the pupal period. If we allow for the pupal skins, the increase in chitin is about 12 gm. per 1000 individuals.

Heller<sup>(17)</sup> has used Kellner's figures for the purpose of calculating the energy used in metamorphosis, but it is not clear that his use of the chitin data is justified. He assumes that the whole of the chitin content (20.82 gm. per 1000 gm.) of the silkworm is lost in the moult, the 8.47 gm. of chitin in the pupae being of new formation. Similarly he assumes that the whole of this is lost in the pupal skin, the chitin of the moth being entirely new. What exactly Kellner, Sako and Sawano did with the larval and pupal skins is by no means clear from their paper, but they certainly did not use the figures in the same way as Heller.

According to Kellner and Inouye, then, the total new formation of chitin in metamorphosis (the excess of chitin in the moth over that in the pupa, plus the chitin of the pupal skins) is 12 gm. per 1000, containing 0.84 gm. of nitrogen. This nitrogen is probably derived from protein.

We have now a description of the chief metabolic changes suffered by *Bombyx mori* during metamorphosis, and may go on to consider and to compare with this description the chemical studies which have been made on other types of insect.



We may take first other examples from the Lepidoptera. Heller<sup>(17)</sup> made a very thorough study of the hawk-moth *Deilephila euphorbiae* in order to compare this form, which spins only an insignificant cocoon, with *Bombyx mori*. Describing the beginning of pupation, Heller says, "During the first period (*i.e.* during the first four days after the worm ceases to eat) the worm moves about almost continually and gives out 262 mg. of water daily. Then it lies motionless, only from time to time spinning a thread round itself, until in three or four days a loose cocoon is ready. This is a period of such great excretion of water that the walls of the respiration-chamber become wet. In the last three days the spindle-shaped body of the worm lies without movement until moulting, and the excretion of water diminishes. Altogether in the last eight days 1.43 gm. of water was given out." The following interesting tables are given by Heller:

Mg./1 individual	Composition of			Loss during formation of	
	Larva	Pupa	Imago	Pupa	Imago
Wet weight ... ..	4038.0	2608.8	1263.0	1430.0	1345.8
Dry weight ... ..	848.0	652.0	400.5	196.0	251.5
Water ... ..	3190.0	1956.8	862.5	1234.0	1094.3
Fatty acids ... ..	141.2	99.7	77.1	41.5	22.6
Nitrogen ... ..	62.2	57.6	42.3	4.6	15.3
Crude protein ... ..	378.5	348.0	249.0	30.5	99.0
Chitin ... ..	27.2	34.0	39.6	27.2	34.0
Ash ... ..	36.3	30.6	24.0	5.7	6.5
Non-nitrogenous extract	264.0	141.2	11.6	122.8	129.6

It will be observed that during formation of the pupa *Deilephila* loses less than one-quarter of its dry weight, instead of one-half as does *Bombyx*, also only about one-third of its protein instead of nearly two-thirds as in *Bombyx*. During the pupal period *Deilephila* uses a larger proportion of protein and a smaller proportion of fat for combustion purposes than *Bombyx*, but like *Bombyx* it uses nearly the whole of its store of carbohydrate.

Heller also measured the oxygen-consumption and the CO<sub>2</sub>-output at various stages, and thus was able to calculate the respiratory quotient (R.Q.). This value worked out at 0.88 during the first four days after feeding had stopped, a period during which the

mean oxygen consumption was 40 c.c. per day. Then there followed a period of five days when the R.Q. was 0.76 and the oxygen consumption 10 c.c. per day. During the last three days before moulting the daily oxygen consumption rose to 24 c.c. and the R.Q. to 0.87. After the formation of the chrysalis the oxygen consumption dropped to 4 c.c. per day and the R.Q. for the first few days to 0.75—

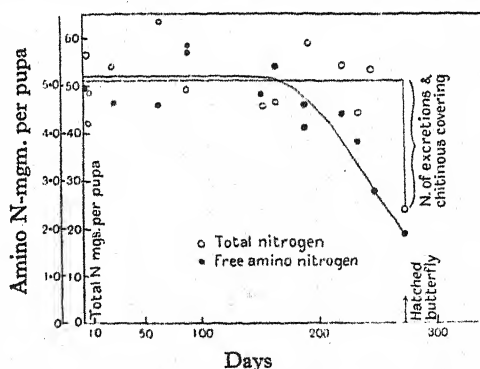


Fig. 5. *Deilephila*. Heller.

thus the very marked lowering of respiratory activity noted in *Bombyx* after chrysalidation occurs here too.

In another paper (15) on the same insect Heller followed the total nitrogen and the amino-nitrogen by Van Slyke's method all through metamorphosis, thus obtaining some of the data the absence of which we had to lament in the case of the silkworm (see Fig. 5).

	Age in days from cessation of feeding	Amino-nitrogen in % of the total nitrogen
Tissue still intact ... ..	1	8.78
Disintegration starting ...	6	11.78
Disintegration complete ...	26	8.59
" " " " " " " " " "	53	10.09
" " " " " " " " " "	67	8.73
" " " " " " " " " "	91	11.75
" " " " " " " " " "	155	10.53
" " " " " " " " " "	163	11.74
" " " " " " " " " "	—	9.93
" " " " " " " " " "	191	7.64
" " " " " " " " " "	—	8.08
First outline of digestive tube	—	8.63
Distinct contour of moth ...	—	5.11
Complete moth ... ..	273	7.99

Very similar results were obtained by Courtois (10), who used extracts made with warm water, precipitated the proteins with trichloroacetic acid, and then titrated with formol according to Sørensen's method. Nessler's reagent showed that only traces of ammonia were present.

	Mg. amino- nitrogen %
<i>Saturnia carpi</i> Jan. 7	250
Jan. 11	245
Jan. 16	250
Feb. 28	231
Mar. 1	126
Mar. 7	122
<i>Attacus cynthia</i> Feb. 20	250
Feb. 25	245
Mar. 7	180
<i>Attacus pernyi</i> Mar. 7	232
Mar. 10	222
Mar. 19	175
<i>Sphinx pinastri</i> Feb. 15	250
Feb. 25	241
Apr. 10	178

The weights of individual pupae are not here given, but if we assume that the pupae of *Sphinx pinastri* are of approximately the same weight as those of *Sphinx* (or *Deilephila*) *euphorbiae*, we see that the results of Heller and of Courtois are in satisfactory agreement.

Heller remarks that during the metamorphosis of *Deilephila* the interior of the chrysalis is "reduced to a semi-liquid mass." This disintegration, however, is not accompanied by any striking change in the amino-nitrogen, as is evident from the

above figures, which remain more or less constant from the first to the 191st day. It is true that at the time when the outlines of the moth appear the amino-nitrogen definitely falls. Similarly Courtois observed that the amino-nitrogen is constant until the organs of the moth appear at the opening of the chrysalis, when there is a diminution.

Courtois<sup>(11)</sup> has also investigated the changes in the amount of cholesterol and fatty acids present during metamorphosis, with the following results:

Animal	Fatty acids % wet weight	Unsap. sub. % wet weight	Unsap. f. acids	Cholesterol % wet weight	Lipocytic coefficient chol. f. acids $\times 100$
Pupa of					
<i>Attacus pernyi</i>	4.64	0.8	17.2	0.07	1.7
<i>Sphinx ligustri</i>	7.82	0.4	5.3	0.09	1.2
<i>Saturnia pini</i>	8.19	1.4	17.0	0.09	1.0
For comparison:					
Adult of					
<i>Hirudo medicinalis</i>	—	—	—	—	20.0
<i>Ostrea edulis</i>	—	—	—	—	7.3
<i>Asterias glacialis</i>	—	—	—	—	9.2

The lipocytic coefficient is thus seen to be remarkably small in the pupating Lepidoptera, a fact which probably may be associated with the comparatively dry nature of the system as a whole.

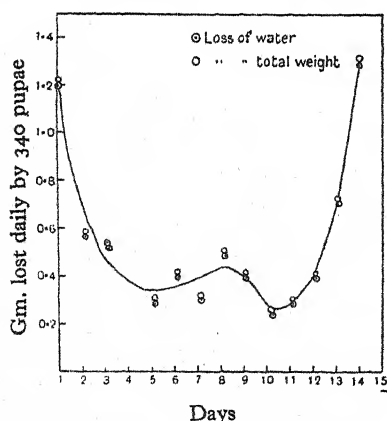


Fig. 6. *Calliphora* pupae.  
Weinland.

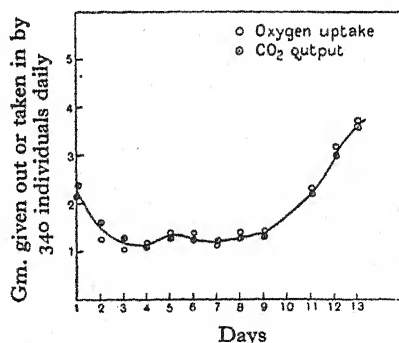


Fig. 7. *Calliphora* pupae. Weinland.  
Gaseous exchange.

Some good weight curves for *Phalaena pavonia minor* are given by Urech<sup>(36)</sup>.

Shinoda<sup>(29)</sup> in 1926 published a paper on *Dictyoploca japonica*, the wild silk-moth. In this case also we can see the loss of weight on pupation and the increase in dry weight, as well as the piling up of fat about the time of spinning and its subsequent disappearance. His figures for glycogen were very small and irregular.

Leaving the Lepidoptera, we may turn to the extensive studies made by Weinland on the blow-fly, *Calliphora vomitoria*, one of the Diptera.

The pupation period in *Calliphora* is about fourteen days; at the beginning of this time the larvae stop feeding and seek out a sheltered spot in the dark, where they settle down, contract in length and remain motionless. No cocoon is made but the last larval skin is retained as a barrel-shaped puparium. If a pupating larva at this early stage is thrown into boiling water, it lengthens and resumes its former shape. But if the experiment is made a day or two later the larva remains contracted; this is due to the histolysis of the larval muscles which begins in the first hour or two of pupation; while this breakdown is going on the tissues of the adult are being built up from the imaginal discs. Curves are given for the carbon dioxide and water output and for the loss in weight and the oxygen intake (Figs. 6 and 7). In  $\text{CO}_2$ -output and weight loss they resemble those for *Bombyx*. As in *Bombyx* and in *Deilephila*, there is in the early stages a very marked excretion of water, and drops of water collect in the respiration chambers. From the oxidation curve it is clear that only a very small proportion of this water can be produced by respiration. This latter curve runs much the same course as that for  $\text{CO}_2$  elimination, but rises a little more rapidly at the end. The following figures are given by Weinland (38) for other constituents:

	Gm. per 1336 mature larvae	Gm. per 1336 pupae	At the end of metamorphosis (gain or loss)
Weight ... ..	100.0	85.0	-15.0
Petrol-ether extract	6.96	3.93	-3.03
Glycogen ... ..	0.63	0.17	-0.46
Chitin ... ..	2.21	3.24	+1.03
Total nitrogen ...	3.15	3.22	+0.07

It would appear from this that the greatest part of the energy requirements must be met by combustion of fat. This is borne out by the R.Q. in some experiments where average values of 0.71 and 0.68 were obtained; on other occasions very low quotients were found, for which some special explanation must be sought.

It seems probable that all the glycogen disappearing goes towards the formation of the new chitin, which bulks more largely in the balance-sheet of *Calliphora* than in that of *Bombyx*. If we assume this, there remains only 0.57 gm. of new chitin unaccounted for in 100 gm. of pupae. This, as we shall see, probably came from protein. Weinland found that the total nitrogen content remains constant during pupation, so that, as in the case of *Bombyx*, no volatile nitrogenous compounds are given off. The nitrogenous end-products remain in the pupa, and after the emergence of the fly are excreted by the latter as a material which quickly dries to a whitish powder and gives all the tests for uric acid. The amount of the excreted nitrogen could not be determined with great accuracy, but it was found to be at least 0.64 gm. per 100 gm. of pupae. As the nitrogen of the newly-formed chitin was 0.7 gm. the total nitrogen which had undergone metabolism was about 1.34 gm., corresponding to about 8.04 gm. of protein. This would be ample to account for the chitin produced in excess of the glycogen disappearing.

Unfortunately Weinland did not determine any of the nitrogen fractions other than the uric acid. But he performed many interesting experiments, using a *Brei* of pupae<sup>(39,40)</sup>. Shaken in oxygen at room temperature, the fat content of such a *Brei* showed a marked decrease, much greater than would have been shown by the intact pupae. CO<sub>2</sub> was given off, but in much smaller amounts than would correspond to complete combustion of the fat; also some unidentified volatile substance seemed to be formed. Besides fat, lecithin was apparently broken down; this was deduced from the fact that the petrol-ether extract contained much less phosphorus after the oxidation than before. If the *Brei* was shaken anaerobically Weinland found that CO<sub>2</sub> and H<sub>2</sub> were given off. In the pupa *Brei* three processes<sup>(41)</sup> could be discerned in which carbohydrates were involved:

1. Formation of carbohydrate.
2. Disappearance of carbohydrate.
3. Formation of chitin.

The intensity of the first-named process seemed to be entirely dependent on the amount of carbohydrate already present in the *Brei* at the beginning of the experiment. If this was already at the maximum of 300 mg. per 20 gm. of *Brei*, then no further carbohydrate formation would take place. The source of the carbohydrate was probably protein, for chitin was never found to decrease at the same time, and the diminution in fat bore no quantitative relation to the increase in carbohydrate. Chitin formation took place most readily when the *Brei* was not shaken and when much carbohydrate was initially present; the process was always accompanied by loss of carbohydrate.

Just after this work of Weinland's, Tangl<sup>(34)</sup> published an extensive investigation of *Ophyra cadaverina*, the corpse-fly, which included results very similar to those on *Calliphora*. The following table summarises his analytical data. On

Gm./100 individuals	Water	Weight		Org. subs.	Ash	Fat (petrol-ether ex.)	Nitrogen
		Wet	Dry				
Larvae on Apr. 19th	9.21	13.33	4.12	3.87	0.25	1.87	0.29
Pupae on Apr. 25th	5.55	9.30	3.75	3.49	0.26	1.53	0.29
Used in 6 days up to pupation	3.66	4.03	0.37	0.38	—	0.34	—
I.e. per day	0.61	0.67	0.06	0.06	—	0.06	—
Pupae on Apr. 25th	5.55	9.30	3.75	3.49	0.26	1.53	0.296
After 13½ days Cases	0.05	0.95	0.90	0.77	0.13	0.09	0.098
Flies	4.88	7.32	2.44	2.33	0.11	1.03	0.191
Both	4.93	8.27	3.34	3.10	0.24	1.12	0.289
Used in 13½ days	0.62	1.03	0.41	0.39	—	0.41	0.007
I.e. per day	0.046	0.076	0.030	0.030	—	0.030	—

April 19th the larvae were ready to metamorphose and had ceased to feed. A few pupated on the 21st and practically all had done so by the 25th. The marked loss of weight, due especially to water, in the pupation stage, is seen here once more. Tangl did not estimate carbohydrates, but he demonstrated the use of fat during

metamorphosis and also the fact that no nitrogen is lost from the system. About 0.1 gm. of nitrogen per 1000 individuals was found in the pupal cases after emergence, and probably represents most of the waste-nitrogen of the period.

Of other work on members of the Diptera there seems to be little. Sosnovski<sup>(31)</sup> studied the carbon dioxide output of the pupae of *Musca vomitoria* and of *Lucilia caesar*. He obtained curves very similar in shape to that for *Calliphora*, but omitted to give in his paper any weights or volumes. Then there is the isolated work of Jezevska<sup>(20)</sup>, who followed the total tryptophane content of the pupae of *Musca vomitoria* during their metamorphosis. For the first two days there was a fall, but this was followed by a very marked rise to a maximum (at 140 per cent. of the original) on the fifth day. After this there was a falling off. There would thus appear to be a synthesis of tryptophane during metamorphosis, but the actual amounts of tryptophane per 100 pupae or per gm. of pupa cannot be discovered from Jezevska's paper.

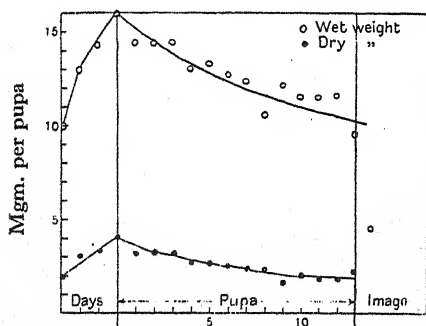


Fig. 8. Weight loss in worker-bee pupa. Straus.

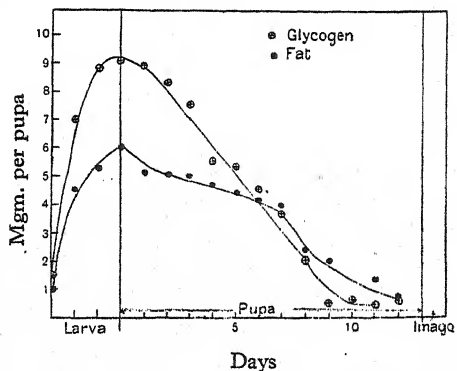


Fig. 9. Pupa of worker-bee. Straus.

We come now to the Hymenoptera.

For the honey-bee, *Apis mellifica*, we have the data of Straus<sup>(33)</sup>. His curves (see Figs. 8 and 9) show the changes in wet and in dry weights, and in glycogen and fat, for the workers. On the sixth day of larval life the cells in which the larvae are developing are closed off by the worker-bees with a lid. Soon after this the spinning begins and a complete cocoon attaching the larva to the walls and the lid is formed. It is unknown when the last moulting inside the cell takes place. There is often a little food remaining in the cell when it is closed and the metamorphosing larva may have food material in its intestine up to the third day after closing, but for convenience the closing of the cell is taken as the first day of pupation. By the end of the third or fourth day the larva begins to change its shape, and in the following two days the transformation goes on very rapidly, so that by the end of the sixth day the whole imago is to be seen quite without pigment. Pigmentation begins on the seventh day, and on the twelfth the imago emerges.

First of all, there is to be noted a striking difference from any of the types already



studied in that the percentage of dry weight falls during pupation. The wet weight falls abruptly during the first and second days by about 18 per cent., and at the same time the dry weight falls by about 20 per cent. The work of spinning is probably concerned with this, as well as the fact that during this time there may be evacuation of the intestine and the last moult. Very much less relative loss of weight takes place during this period than with *Bombyx*, where the cocoon is much larger and spinning takes much longer. By the twelfth day the wet weight has fallen by 35 per cent. and the dry weight by 57 per cent. of the original values. The bee pupa is, of course, protected from water loss by its position in the closed cell.

By the eighth day of pupation almost 60 per cent. of the glycogen has disappeared, and only about 26 per cent. of the fat. After this time both fat and glycogen disappear till both are reduced almost to zero. Between the third and the twelfth day the chitin content rises from 30 mg. to 90 per 100 individuals. On the whole the total nitrogen remains constant, and no other nitrogen fractions were estimated. This description is for the pupa of the worker; the course of events in the drone pupae is very similar.

Bishop<sup>(6a)</sup> and Bishop, Briggs and Ronzoni<sup>(8)</sup> have also studied metamorphosis in the bee, but from a different point of view. Bishop found that the oxygen capacity of larval blood was equal to the amount of oxygen which could be physically dissolved, i.e. that there was no oxygen carrier present. During spinning the oxygen content decreased, for the larva is enclosed in its cocoon, and although the diffusion is cut down the demand has increased. He also found that the  $\text{CO}_2$ -absorption curves indicated that the blood (of drone larvae) decreased in  $\text{CO}_2$ -capacity and content during spinning, but the tension of  $\text{CO}_2$  and the hydrogen-ion concentration increased, giving evidence of loss of alkali reserve through the production of acids other than  $\text{CO}_2$ . After spinning the pupal blood showed a decrease of  $\text{CO}_2$ -tension and content, with little change in capacity, allowing the pH of the blood to return to its normal level.

Perhaps the most striking biochemical fact of metamorphosis is the U-shaped curve which invariably appears when the  $\text{CO}_2$ -output or the  $\text{O}_2$ -uptake is studied. A few such curves have been reproduced, but many others may be found in the literature—for example, those of Krogh<sup>(25)</sup> for *Tenebrio molitor*, Sosnovski<sup>(31)</sup> for *Musca vomitoria* and *Lucilia caesar*, Bodine and Orr<sup>(9)</sup> for *Drosophila melanogaster*, Taylor<sup>(35)</sup> for *Phormio terenaevae*, *Phormio regina* and *Lucilia sericata*, Tangl<sup>(34)</sup> for *Ophyra cadaverina*, and Fink<sup>(12)</sup> for *Leptinotarsa decemlineata*, *Hippodamia convergens*, *Hylemia cilicrura*, *Ancylys comptana*, *Popillia japonica*, *Crioceris asparagi* and *Macrocentrus ancylovora*. It is likely that it is the shape of this curve which has been mainly responsible for the tendency among workers on the chemical aspect of the subject to assume the validity of the old view of more or less complete degeneration to a formless pulp, followed by the construction of the adult organs. Thus Krogh<sup>(25)</sup> expressed the hope that it might be possible to show a quantitative relation between the amount of organised tissue present and the gaseous metabolism. Heller<sup>(16)</sup> also wishes to distinguish three stages in metamorphosis. The first is that of histolysis, or the transformation of the tissues of the larva into reserve

material, accompanied by a considerable lowering of the respiratory exchange. The second stage is characterised by a stability of the metabolic intensity established at a low level. In the third stage is placed the reconstruction of tissues with formation of the moth, and the gaseous exchange increases more and more during this time. Heller found, using *Deilephila*, that chrysalids which during the early stages of pupation had been kept at 25° showed a lower respiratory exchange (measured subsequently at 18°) than chrysalids which had been kept throughout at 10–18°. He accounts for this by supposing that at the higher temperature histolysis takes place faster and more completely, and thus there is less “organised tissue” to respire during the steady middle period. He concludes that the shortest and most economical metamorphosis could be obtained by exposure to low temperature during the initial period followed by removal to higher temperature, and he did in fact succeed thus in obtaining hatching of *Deilephila* chrysalids in a shorter time than controls kept constantly at the higher temperature. Fink, also, with no attempt at strict morphological correlation, remarks: “The decline in the respiration curves is caused by the very low rates of CO<sub>2</sub>-output during the first few days of histolysis, a concomitant of intense histolysis such as occurs in the pre-pupal state. The general rising of the curve after the initial low phase of metabolism accompanies metamorphosis, the growth of imaginal buds.”

Such conclusions, however, cannot be accepted without further inquiry. It is true that Krogh dissected *Tenebrio* pupae at different stages to obtain evidence of the disintegration and the regeneration, and that Heller observed in *Deilephila* that the interior of the pupa is reduced to a semi-liquid mass “which,” he says, “serves afterwards for the organisation of the imaginal body.” But the very careful histological studies carried out by numerous workers during the last thirty years provide a very different picture. The following emphatic statement is quoted from Henneguy (18). “Another important fact to remember in the metamorphosis of insects, but which may be observed in that of most other animals also, is that the phenomena of histolysis and histogenesis are concomitant. Aristotle and Harvey thought that the larva lost all trace of organisation and in the pupa, so to speak, returned to the state of the egg. Weismann and Viallanes assumed also that the larval tissues underwent complete degeneration and that the new imaginal elements were formed at the expense of the degenerated material. More recent researches . . . have shown that this view was erroneous. When, at the beginning of pupation, there sets in the histolysis of the larval organs destined to disappear or to be transformed, the activity of the histoblasts comes into play, and little by little the organs of the adult are built up, at the same time as those of the larva degenerate and atrophy. There is thus a general gradual transformation of the larval organs into the organs of the imago, and it is only the organs special to the adult which are of new formation, or rather, of simple histogenic evolution from the histoblasts.”

It seems, then, that the histologist would find it difficult to point to any time at which there is present a less amount of organised cell material than at any other. While some organs break down and their débris autolyses or is removed by phagocytosis, other organs are being constructed from the imaginal buds. In some cases,

e.g. certain muscles, the larval organ passes without great disintegration into the imaginal organ, simply losing its differentiation and becoming a homogeneous cell-mass which is then penetrated by the myoblasts. It must be remembered that if tissue previously actively metabolising is becoming degenerate and finally disappearing and not respiring any more, at the same time previously inactive storage material, together with products of breakdown, is being built up into new cells. It is true that during the middle period all or most of the imaginal and larval organs are incomplete and incapable of functioning, and it may turn out that the metabolism is proportional to the amount of tissue present which is capable of functioning, even though the organs are quiescent. Tangl, working on *Ophyra*, was inclined towards the view that diminishing muscular activity in the larvae ready to pupate was the cause of the diminishing oxygen uptake and carbon dioxide output, and that the rise at the end of pupation was occasioned by the beginning of muscular movements in the adult. It seems certain, however, that, although it may be important in some cases, actual movement does not often play a large part. But Tangl also suggested that after all movement has ceased, and indeed become impossible owing to the formation of the cocoon or pupal case, the tonus of the larval muscles may still play a part in determining the magnitude of the respiratory exchange, and that as histolysis proceeds this tonus is lost. Similarly the tonus of the newly-forming adult muscles may be gradually acquired. As for the actively dividing imaginal buds, they might be expected to show, like embryonic tissues, a high metabolic rate.

The old controversy as to the method of histolysis, whether by autolysis or by phagocytosis, has little interest for the biochemist, for the digestion-processes will probably be the same in either case. It seems to be recognised now that both methods come into play, autolysis predominating in some cases and phagocytosis in others.

A far more interesting problem is that of the physico-chemical cause of the initiation of metamorphosis. The cause of the onset of histolysis has been variously described. The early supporters of the phagocytosis theory were content to put it down simply to the arrival of an army of phagocytes, ignoring the questions of what stimulated them to activity, and why they should attack certain tissues rather than others. It seems clear, as one considers the progressive nature of histolysis, and the fact that in close proximity to digested tissues other tissues may persist, that some change in the tissue itself must determine the action upon it of the phagocytes.

Later writers have suggested various causes for such changes in the tissues leading either to autodigestion or to phagocytic digestion. Bataillon<sup>(4)</sup> thought that histolysis was conditioned by the suffocation effect of accumulation of  $\text{CO}_2$ , for which accumulation he found experimental evidence in the silkworm, as we have seen. Later, Singh-Pruthi<sup>(5)</sup>, working on the blow-fly, contradicted this hypothesis by showing that added  $\text{CO}_2$  has the effect of retarding rather than accelerating the onset of metamorphosis. He placed the larvae in atmospheres containing 10, 20, 40 and 60 per cent. of  $\text{CO}_2$  (by volume), and found that the retarding effect was proportional to the  $\text{CO}_2$ -concentration. Bishop<sup>(7)</sup> suggested a rise in the acidity of the tissues as one of the causes of the onset of histolysis. He found *in vitro* that

the fat body of the bee larva undergoes autolysis much more slowly than the fat body of the bee pupa, and as a lowered pH is known to favour autolysis, it seemed justifiable to presume a lower pH in the pupal sample. In buffered solutions the tissues from the two stages of development autolysed at equal rates, so there was no reason to suppose any change in the concentration of the enzymes responsible. As was mentioned before, there was evidence for the formation *in vivo* of non-volatile acids and a rise in blood pH during spinning, probably as a product of that activity. When autolysis took place *in vitro* the reaction tended to become more and more acid as the products accumulated; *in vivo*, the pH first falls from 6.85 to 6.65, and then rises again almost to its initial level when the spinning is over. This comparative constancy of the pH is probably due to the using up of the products of autolysis as fast as they are formed.

Glaser<sup>(14)</sup>, in 1925, though accepting Bishop's results for the bee, expressed the opinion that any generalisation as to the correlation of change in pH with metamorphosis would be most unsafe. He found the pH range of the blood of grasshoppers and houseflies to be from 7.2 to 7.6, for *Malacosoma americana* (the tent-caterpillar) to be from 6.4 to 7.4, and for *Bombyx mori* from 6.4 to 7.2, but in these forms no correlation existed between blood pH and age or between blood pH and metamorphosis. Brecher<sup>(9a)</sup> has found the pH of the blood of both larvae and pupae of *Pieris brassicae* to be constant at 6.6 (6.50-6.77) during pupation.

It has also been suggested that hormones may play an important part in bringing on histolysis; this side of the question has been discussed by Koller<sup>(23a)</sup>, who found that injection into young *Sphinx* larvae of blood from larvae settling down to pupate could hasten pupation in the unripe larvae to a very striking degree.

Other less well supported theories have been put forward from time to time, but none so far has reached the centre of the problem and shown clearly by what mechanism one tissue is attacked and an adjacent one left unmolested. High CO<sub>2</sub>-tension must presumably spread throughout the cocoon; it is possible to suppose the production of non-volatile acids in certain definite locations, but before any such hypothesis as this can be proved it will be necessary to bring the technique of Spemann to the attack and to combine it with chemical methods of the utmost delicacy. The elucidation of the stimulus to sudden growth in special parts must also await the application of this refined analysis.

#### SUMMARY.

The alterations in gaseous exchange and in carbohydrate and fat content of the organism during metamorphosis are described. The nitrogen metabolism of this period is considered, the various fractions, protein nitrogen, basic nitrogen, water-soluble nitrogen, chitin nitrogen, uric acid nitrogen, etc., being followed as far as possible. The formation of the silk is considered, and the differences in metabolism between insects forming large and those forming insignificant cocoons.

The question of the physico-chemical causes of the onset of histolysis and of regeneration, and the question of the origin of the low respiratory exchange during the middle period of pupation, are discussed.

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# THE PROBLEM OF SPECIES IN VIEW OF THE ORIGIN OF SOME NEW FORMS IN MICE

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(Received May 31, 1929.)

(With Plates VIII-XI.)

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## I. INTRODUCTION.

THE discovery of X-rays and the radio-active substances has placed a new tool in the hands of the student in different fields of biology and has given a great stimulus to investigations undertaken with a view to changing artificially the germ plasm of an animal. The modifications produced by the rays in the isolated sperm, in the testicles, in the oocyte and the ovaries, as well as their bearing on fertilisation and embryonic development, have been much studied, and many points have been elucidated with a reasonable amount of supporting evidence. These investigations were soon followed by a closer inquiry into the possibility of the hereditary transmission to successive generations of the modifications produced in the germ cells by radiation. As I have already cited in detail in my previous papers many publications concerning all these questions, I shall now briefly run over only a few recent works, showing that opinions concerning the hereditary behaviour of the irradiated germ plasm are still very divided.

Bagg and Little (1923-1924) irradiated a series of mice, 20 males and 10 females, and the animals were given 12-second exposures on each of five successive days. The mice were confined in a small space directly beneath the X-ray tube and the entire dorsal surface of the body was irradiated. The physical conditions were as follows: target-skin distance 12 inches,  $2\frac{1}{2}$  inch sparkgap, current 10 milliamperes, no filter. The first litters were obtained 13 and 14 weeks after irradiation.



Of the 20 treated males, 14 gave no descendants. They were either sterile or they died. Three of the treated females died and left no descendants. Of the remaining 7 females, 3 were mated with 3 different males and the other 4 females were divided into groups of two, with a different treated male for each group. Two treated males were mated with untreated females. The offspring of the first generation were all normal; abnormal-eyed animals were found in the third and subsequent generations: two matings, each of a different male and female, produced very similar types of abnormality both morphologically and genetically.

The eye abnormality exhibited a wide range of variation, from a slightly perceptible defect in one eye to nearly complete atrophy of both eyes. The eye defect was associated with marked optic atrophy and a deformity of the skull on the same side of the head with defective eye. In a few cases the ear adjacent to the abnormal eye showed a marked reduction in size. In addition to this, many animals have shown the presence of club-feet associated with the abnormal eye condition. The defect mainly affected the soft parts of the foot, but the bones of the metacarpus and phalanges were also slightly reduced in size.

All these abnormalities could be transmitted to successive generations and some of them were proved to behave as a mendelian recessive. Later, kidney abnormalities were also found in the same strain of mice.

The above results led the authors to the conclusion that "this effect appears to be of the nature of a direct effect on the germ-cells themselves and not through the soma as an acquired character."

As the biological factors are generally opposed to such a happening, Bagg (1926) has undertaken, in collaboration with MacDowell, a repetition of the original experiment. The experiments are still being continued, but the results up to the moment of communication have been unquestionably negative, although several thousand animals have been observed.

This last result is paralleled in the experiments of Snyder (1925). The testicles of more than 50 adult male rats were X-rayed under the following physical conditions: 65,000 volts, 10 milliamperes, 40 cm. target-skin distance. The time of exposure alone was varied from one minute to an hour and a half. The first litter after sterility period, considered as critical, was inbred by brother-to-sister matings for subsequent generations. A total of 1024 individuals was obtained, and the sixth filial generation was reached with no sign of abnormality of any kind, no apparent reduction of vigour and no decrease in fertility.

Mavor began his work with X-raying of *Drosophila melanogaster* in 1921. Four series of experiments have since been carried out. In the first series, wild-type (red-eyed) virgin females were X-rayed soon after emerging from the pupa with a dose just under the sterilisation dose, and mated with white-eyed males. In the second series, white-eyed virgin females were X-rayed and mated with eosin-eyed miniature-winged males. In the third and fourth series, virgin females resulting from the cross white-eyed normal-winged female by eosin-eyed, miniature-winged male were X-rayed and mated to wild-type males. In the first and second sets of experiments a Coolidge portable outfit was used and the flies were X-rayed

in glass cups placed on an insulating glass plate close to the X-ray tube. In this case the flies were exposed to a strong electrostatic field as well as to the X-rays. In the case of the third and fourth sets of experiments the X-ray treatment was given to the flies with the apparatus described by Davey. The flies were placed in the small glass cups, covered with a single thickness of "onion-skin" paper, which was the only material intervening between the flies and the glass of the Coolidge X-ray tube. The Coolidge tube was kept at 50,000 volts; the number of milliamperes passing through the tube, the distance from the target and the time of treatment were different in different experiments.

The results of these experiments, which it would take too long to describe here, have led the author to the assumption that the effect of the X-rays is to produce non-disjunction of the sex-chromosomes.

This phenomenon was first recorded in normal conditions by Bateson and Punnett (1911) under the name of "reduplication," and afterwards described by Bridges (1916) in *Drosophila* as proof of the chromosome theory of heredity; later, it was noticed by Mohr (1919) during his cytological study of the testicles in irradiated *Decticus verrucivorus*.

When the non-disjunction occurs in *Drosophila*, two kinds of exceptional eggs are formed, one kind without any X-chromosomes, and the other with two X-chromosomes. Exceptional males are formed when an egg without any X-chromosome is fertilised by an X-chromosome bearing sperm and they have the sex-linked characters of their father. Such is the case with the exceptional males (white-eyed) obtained in the experiments of Mavor. Such males when they are found to occur naturally are sterile, a condition found also in the exceptional males produced by the X-rayed mothers. Exceptional females are formed when an egg with two X-chromosomes is fertilised by a Y-chromosome-bearing sperm and they have the sex-linked characters of their mothers. The exceptional females are fertile and produce further exceptions, as in the case of non-disjunction occurring naturally.

The effect of the X-rays, so far as the occurrence of these exceptional flies is concerned, can be demonstrated by a general summary of the production of non-disjunction in these experiments. The 26 X-rayed females produced a total of 1944 regular sons, 42 exceptional sons, 2173 regular daughters, and 8 exceptional daughters. The 19 control females produced a total of 5119 regular sons, 3 exceptional sons, 4985 regular daughters, and one exceptional daughter. The exceptional males tend to occur at one of two specific periods, namely, either 11 to 12 days after X-raying or 16 to 18 days after this, these times corresponding to eggs laid on the second and third days or the fifth to ninth days after X-raying. It would appear therefore that the formation of the exceptional males is due to the X-rays acting at a particular period in the maturation of the egg and causing the elimination of the X-chromosome. On the other hand, the times of occurrence of the exceptional daughters did not show so clear a grouping into the periods, although there was some evidence of such a grouping.

The author has also studied the effect of X-rays on the linkage of mendelian

characters in the first and the second chromosomes of *Drosophila melanogaster* and has shown that X-ray treatment of the female leads to a decrease in the cross-over value between eosin and miniature (first chromosome) and to an increase in the crossover values for black to purple and purple to curved (second chromosome).

In a note upon "The attack on the gene" Mavor (1925) arrives, however, at the following conclusion: "In spite of these peculiar and rather profound effects on the mechanism of the inheritance of unit characters, no conclusive evidence has been obtained that any individual gene or unit character has been altered, although during the course of experiments somewhat over one hundred thousand flies developed from X-rayed eggs have been studied." This conclusion seems to be in flagrant contradiction with the results obtained during the last two years.

Muller (1927) read a paper on "The problem of genic modification" before the Fifth International Congress of Genetics in Berlin, and later (1928) another one "The production of mutations by X-rays" before the National Academy of Sciences. As the visible mutations in *Drosophila* were found to be extremely rare—scarcely one mutant among 50,000 flies—the author uses in this work more frequent lethal mutations as indices of gene mutation in general. These lethal mutations may be regarded as commonly differing from visible mutations only in the more drastic end-result which they happen to produce upon the organism. The influence of X-rays on the rate of mutations was studied on the flies containing a different collection of genes in their X-chromosomes, the males having bobbed (though appearing normal, as bobbed does not show in the male), and the females scute, vermilion and forked. In most cases only one parent was subjected to the X-ray treatment, which was given under the following physical conditions: broad-focus Coolidge tube with tungsten target, at a distance of 16 cm. from this target and with an aluminium filter 1 mm. thick. The peak kilovoltage was 50, the milliamperage 5. Four different lengths of treatment were given to the males, of 12, 24, 36 and 48 minutes' duration respectively, and these treatments were designated as t-1, t-2, t-3 and t-4 respectively. The treated females were given only t-1 and t-2 treatments. Directly after the treatment, six series of cultures, each series containing from 25 to 30 cultures, were established. When the  $F_1$  flies hatched, 81 having distinguishable (though often slight) morphological abnormalities were observed among somewhat over 2000 flies examined; among about the same number of controls there were only 19 that seemed correspondingly abnormal. "Among these abnormalities—remarks the author—there must of course have been included modifications not genetic in their basis."

The  $F_1$  flies, not necessarily virgin, were then mated together, brothers to sisters as far as possible. In the  $F_2$  generation the controls showed 1 lethal mutation in 947 fertile cultures, and the treated series 88 lethals in 758 cultures, and all but 3 of these lethals were confined to the chromosomes derived from the treated progenitor. Similar results were found with regard to visible mutation and semi-lethals in these cultures, though they were not as numerous as the lethals. The results were substantially the same in a later set of experiments (somewhat over 1600 fertile cultures), similar in principle to the preceding. On adding together

the data of both sets of experiments, the author finds the lethal mutation frequency to be of the order of 150 times higher in the heavily treated t-4 series, than in the controls, and also significantly higher in the t-4 than in t-2 lots.

To obtain more abundant data on visible mutations, the treated males were crossed with females having attached X-chromosomes and a Y-chromosome. Here the sons carry their mother's Y and their father's X and hence reveal all visible mutations, even recessives, that arose in this X of the treated sperm.

The abnormalities thus produced were very abundant, and similar results were obtained later on repetition of this experiment. The induced mutations in the X-chromosome were heaped more abundantly in the same regions as those in which a greater number of spontaneous mutations had previously been found. The data on dominants indicate that mutations are induced as readily in the autosomal chromatin as in that of the X-chromosome. Numerous cases of the reorganisation of chromosomes, so called "chromosome mutations" were also found. The majority of the induced mutations seemed to be the same as, or allelomorphic to, already known spontaneous mutations in *Drosophila*.

To finish this review of the very interesting works of Muller, we shall quote the words of the author himself. In the first of the two papers referred to above, he says: "It appears quite clear, from these statistics, that mutations are produced by X-rays in *Drosophila*, and furthermore, that they are produced both by treatment of the mature sperm, and of the eggs." And in the second: "Thus we are brought before the question: are all mutations ultimately due to rays of short wave-length and to high-speed particles of corresponding energy content? If so, biological evolution has been made possible only by the stray radiation present in nature—the beta and gamma rays, and the cosmic rays."

The following students in the same field, using mostly the same experimental methods, furnish evidence closely approaching the above mentioned investigation.

Hanson (1928) treated the males only of *Drosophila melanogaster*, with the doses known in Muller's laboratory as t-2 and t-4, and crossed these treated males to untreated females. The  $F_1$  generation presented the following mutation rates, using the word mutation loosely to include gene mutations, chromosome mutations and somatic mutations: t-4 group gave 35 mutations and 1077 normal flies, i.e. 0.035 mutation rate; t-2 group gave 15 mutations and 2427 normal flies, i.e. 0.006 mutation rate; and the controls 3 mutations out of 5007 flies, i.e. 0.00059 mutation rate. A large percentage of the flies showing these mutations were sterile. The paper was published at the moment when such as were not sterile were being bred in various combinations to determine the nature of the changes.

In another work, in co-operation with Heys (1928), the same author studied the effects of radium in producing lethal mutations in *Drosophila melanogaster*. The experiments established: in the first case, 4 mutations in 31 tubes with treated flies, and no mutations in 110 control tubes; in the second case, 35 mutations in 426 tubes treated with unfiltered radium rays (140 mg. during 6 hours); and 12 mutations in 426 tubes treated with gamma rays only (150 mg., 13 hours, 2 mm. of lead). At the same time, 423 control tubes yielded no mutation.

Weinstein (1928) exposed males of *Drosophila melanogaster* to t-4 and t-2 doses (Muller) and mated them afterwards to untreated females. Nine of 37  $F_1$  females in the t-4 series, and 10 of 47  $F_1$  females in the t-2 series were found to have inherited altered X-chromosomes from their fathers. Mutations were obtained producing visible and lethal effects, as well as genetic modifications in the frequency of crossing-over and attachments between genes of different chromosomes; six such cases of translocations were found, in which genes of the second chromosome behaved as if attached to the X-chromosome, that is, they were inherited in sex-linked fashion.

Patterson (1928) studied the effects of X-rays in producing mutations in the somatic cells of *Drosophila melanogaster*. Irradiations of 25 and 50 minutes, at a target distance of 12 cm., 50 kilovolts and 5 milliamperes, were administered to the  $F_1$  eggs and larvae obtained from crosses between the normal red-eyed flies and the white-eyed mutants. The object of making this cross was to get females heterozygous for the sex-linked genes. Since the male of *Drosophila* has but a single X-chromosome, his somatic cell will have either the dominant (red-eye) or recessive (white-eye) gene, but not both. The 217 females from treated larvae, homozygous for the sex-linked genes, showed no change in eye colour; the 666 females from treated larvae and heterozygous for this gene, showed 24 separate white areas (either single or groups of white ommatidia); the 807 males from both treated series gave 8 males showing white areas. The controls gave 1798 flies, of which 991 females and 807 males, all red-eyed.

Test for gene mutations: 12 control females gave 1732 offspring among which no visible mutation was found. Fourteen of the 29 females from treated larvae were fertile and gave 1861 offspring, of which 5 showed visible mutations. Twenty-three of the 44 tested males were fertile and gave 944 male offspring, of which 20 showed visible mutations.

Analogous results were obtained by Whiting (1928) on *Habrobracon*, by Stadler (1928) in barley, by Goodspeed and Olson (1928) on *Nicotiana* species, etc., Nadson and Philippov (1925 and 1928) differentiated, by means of X-raying, several new races in *Mucorinae*.

It is evident from the foregoing that the results obtained with the penetrating rays on different species are far from being conclusive, and that even in *Drosophila* the most divergent opinions may be encountered.

## II. PERSONAL OBSERVATIONS.

In 1923 Professor Regaud directed my attention to the desirability of studying the influence of the modifications produced by the X-rays in the testicles, on heredity in mice, and I have since been pursuing this inquiry.

In order to secure the exact data regarding the lesions produced by the different doses of X-rays in the male germ cell, I have studied microscopically the testicles of 46 mice irradiated in very different conditions and operated first on one and later on the other side (or taken when the animal was dead) at different periods after

irradiation (a detailed record is published in *Archives d'Anatomie microscopique*, 1927). The importance of morphological changes produced has been thus established. Meanwhile, 42 of these irradiated males were mated to different normal females, but only 30 of them were fertile; 12 others proved to be definitely sterilised though many of them lived for a long period after irradiation and were coupled with many females. In addition to this, two more males and three females were irradiated in as nearly as possible the same conditions as the animals of Bagg and Little. These animals were also bred from many times.

Though monstrosities of the spermatozoa and many disturbances in spermatogenesis have always occurred after irradiation in testicles of our mice, the results of the majority of these breeding experiments were absolutely negative through three or four generations (about 3000 mice). The detailed account of these experiments has already been published (*Archives de Biologie*, 1928), and we want to give here only a short summary of the abnormalities obtained. They were not numerous and presented two different groups (a) abnormalities observed only as phenotypic modifications. These are: a case of partial non-ossification of the skull in a young animal of 3 weeks, and a case of brachydactyly and eye abnormality in another young animal. Both died early and their genetic study could not be made. (b) Only two males, apparently quite normal, irradiated with rather moderate doses have given origin to two abnormalities which proved to be hereditary and since have been cultivated in two particular strains.

These two males were exposed to the X-rays produced by a Coolidge tube with tungsten target, at a target-skin distance of 29 cm.; the milliamperage 4, the sparkgap 30 cm. and filtration through 4 mm. of aluminium. The doses were measured on the skin surface with the ionometer of Solomon and expressed in French unit *R*. The testicles alone were X-rayed, the rest of the body being protected by a screen of lead, 8 mm. thick.

The first of these two males, No. 10, gave rise to an abnormal offspring after an irradiation of 50 minutes and a dose of 1024 *R*. This abnormal offspring was a male of the second generation; at the age of 4 weeks, he was found to shake his head in different directions, nearly the whole time. In spite of this, he developed normally, was mated to many normal females and later to his daughters, also apparently normal. These last matings resulted in the appearance of several individuals, males and females, manifesting not only the abnormal shaking of the head, similar to their father, but also rotative movements in the fashion of the real Japanese waltzing mouse. Just as in these latter, this abnormality in our mice breeds true as a mendelian recessive.

The second male, No. 17, was X-rayed three times with doses of 230-233 *R*; the exposure lasted 7 minutes, with the tube on air, and 15 minutes when the tube was immersed in oil. Immediately after each irradiation this male was crossed with normal females, and these crosses resulted in the appearance of several young with kinky tail. In the second generation of the two matings, there appeared, after a second irradiation, four offspring with short tail and one with the tail kinked in the middle and having the end sloughed; this phenomenon has already been



described under the name of "non-viable character." In subsequent matings of these mice tailless offspring were also found.

We have cultivated these two abnormalities and now we possess two special stocks of mice: first that of waltzing mice, which breed true and give no other abnormality, and second that of tailless and short-tailed mice, which are all heterozygotes, *i.e.* they always give disjunction in the first generation, with return to the normal type. Nevertheless, being dominant, the character "abnormal tail" returns constantly in all matings, and indeed many new mutations have been observed, such as filiform tail, a fine skin appendage, 10-14 mm. long, without any sign of skeleton; kinky tails or tails ending in all kinds of hooks; the mutations of this kind depend on unilateral insufficient growth of a vertebra or on an ankylosis between them; interruption of the bony skeleton resulting from suppression of embryonic development of a vertebra; mutations of helicoid type—tails in form of a snail or a helix etc. (see Plates VIII-XI).

The character "abnormal tail," including all particular forms mentioned above, behaves always as a lethal, in this sense that it is unknown in a homozygous state. This condition manifests itself in a diminished number of offspring. In the crosses of tailless to tailless mice, we have obtained (in co-operation with N. Kobozeff) 100 offspring with abnormal tail (70 anoures and 30 brachyures) and 47 normal, *i.e.* nearly 2 : 1 ratio. This is exactly the same proportion which was established by Cuénot (1905) and by Castle and Little (1910) and repeated since by many others in mating of yellow mice. This deficient ratio is explained by non-development of the second homozygous group in the classic mendelian formula 1 : 2 : 1.

In connection with this lethal character of abnormal tail there is, as is generally accepted (Lang, Duboscq), the great difficulty of rearing such animals, due to their great fragility, their slow rate of conception, scanty litters, etc. In spite of all this, established also in our stock, we have succeeded in bringing up many vigorous individuals and good breeders, even in crossings of tailless with tailless which have been considered so far as unfertile.

The evaluation of the rôle of the X-rays in the production of these two abnormalities ("waltzing" and "abnormal tail") in our mice, is a very difficult problem, and it has already been discussed in detail in the paper cited above. Summing up this discussion, we may say that there are several points which argue against the hypothesis of the exclusive causal effect of the rays in our case: first, the small percentage of positive results, only two mutations in 35 breeders and about 3000 offspring. From the other small abnormalities only one case of toe deficiency on a foot might be put down to the rays, but even so it could conceivably be a mere phenotypical modification. The two other abnormalities, closed eye at the age of 41 days (normal eye opening on 14th day) and non-ossification of the skull, were rather an extreme expression of a pronounced condition of rickets. Second, the hereditary abnormalities obtained cannot be regarded as quite new mutations because they have already been found in this locality before: the mice without a tail at all, or with a short tail were observed by Duboscq (those of Lang were also of French origin) and the waltzing mice by Cuénot. The waltzing condition, being

recessive, may be transmitted in a latent state through many generations; and the short tail, though dominant, is occasionally so little different from the normal, that one may fail to identify it. We had actually in our stock a male No. 2, with a tail a little shorter than normal and having a little kink on the end. This abnormality did not attract our attention in the beginning, and this male was also irradiated twice and mated with three normal females. These matings resulted in three litters, and in two of these litters, *i.e.* in the  $F_1$  generation, some frankly brachyure descendants were found. The terminal kink of the tail was also reproduced and so we learn the hereditary significance of this small detail, and the fact that the X-rays were not the only responsible cause for the appearance of the emphasised tail abnormalities found in the progeny of the male No. 2.

This case has brought forward the idea that there might be at large such individuals with lurking tail abnormality in which the ordinary dominant character of this mutation may be concealed by a normal appearance. In connection with this it was supposed that the male No. 17 which seemed somatically quite normal might also have had in his hereditary material a point of deficient stability corresponding to the tail, a kind of predisposition to tail abnormality, which was only unveiled by the rays.

In an effort to obtain more evidence upon the point, we have tried to see if inbreeding alone could furnish the same mutation as has done the X-raying, and we have enlarged our stock of mice with the somatically normal tail, taking our breeders from the ancient stock and also from some new sources. We have now more than three thousand mice, in several pedigree lines, which after careful examination have failed to show any abnormality.

### III. DISCUSSION AND SUMMARY.

#### (a) *Modifications in the germ cells.*

When one sees, on examining the sections under the microscope, the disastrous modifications that the rays produce in a testicle, especially the chromatin changes in the spermatogonia and small spermatocytes, which are transmitted in a hereditary fashion to the subsequent generations of the germ cells, one feels sure that traces of these modifications should be found in the progeny of irradiated animals. The evidence, concerning chromatin changes in natural conditions were summed up by R. R. Gates (1925) in his *Symposium on Species and Chromosomes*; he says that "chromosome changes have arisen in a variety of ways, including transverse segmentation or fragmentation, end-to-end union, gradual diminution and disappearance of certain chromosomes, non-disjunction, rearrangement of portions of chromosomes, crossing of species having different numbers, and polyploidy." The polyploidy has apparently played an important part in evolution of many plant genera and families. Classic examples of this are: (1) *Oenothera gigas*, which is a tetraploid race of *Oe. lamarckiana*, and (2) *Oe. lata*, which has one more chromosome than *Oe. lamarckiana* and is thought to have been formed from the latter by the addition of one chromosome which presumably arose in meiosis through the non-disjunction of one of these elements.

More recently, Blakeslee, Belling and Farnham (1920) have discovered chromosome relationships in *Datura* which parallel many of these previously obtained with *Oenothera*; Täckholm (1920), Blackburn and Harrison (1921), Hurst (1927) on the basis of a very extensive study of polyploidy in different species of *Rosa*, have clarified specification in this genus, and so on.

Since 1910 Morgan and his co-operators have built up a combined system of genetical and cytological evidences in *Drosophila* which has resulted in the sketching out of the maps for gene distribution in the chromosomes of this animal, and in the obtaining of new forms by means of crossing over of these chromosomes. Polyploidy was observed but very rarely in animals.

Chromosome study in mammals is at its very beginning. Painter (1928) has examined the chromosome constitution of Little and Bagg's abnormal-eyed mice. Four males were castrated and the testicles examined. This examination failed to reveal any abnormal chromosome conditions. The spermatogonial chromosome number was 40 in each case (normal diploid number in this animal) and a careful scrutiny of the individual elements, both in spermatogonia and in primary spermatocytes, disclosed no abnormal features.

We have so far examined only the testicles of our irradiated males, and even on them the detailed cytological study is not yet finished. Nevertheless, two most important facts are already well established: inequality of the nuclei in the spermatids and a great number of monstrosities among the spermatozoa. These phenomena were first recorded by Regaud and Blanc (1906) after irradiation of the testicles in rats. They may be accounted for by the irregularities of the two last mitoses and, as we have noticed in our experiments, by the eventual non-occurrence of the last maturation division: cells of the type and dimension of secondary spermatocytes have been seen to change in the same fashion as the spermatids during their transformation into spermatozoa. Furthermore, many among the abnormal spermatozoa have had two heads on their head and, sometimes, the head itself was split into two. As the X-rays produce no new modifications, but only exaggerate what may occur in normal conditions (Regaud), events of this kind might perhaps be responsible for the hereditary doubling of hind legs, tail and external genitalia obtained by Danforth (1925) in mice, and to a certain degree for the polyploidy ("diploide Gameten" of Rosenberg) in plants.

The visible modifications produced by the rays in the germ cells are not definitive if the doses are moderate and do not completely destroy all spermatogonia. When restoration of the spermatogenesis takes place after a period of temporary sterilisation—irradiation having been rather heavy—the first generations of the germ cells present in general visible irregularities in shape, number and disposition. But after a few subsequent cellular generations, complete recovery may be observed and the tubes with restored spermatogenesis and regular disposition of the apparently normal spermatozoa are then seen side by side with completely sterilised tubes.

Such elimination of the induced visible abnormalities by way of successive mitoses suggests the idea that the majority of these abnormalities are only somatic

or phenotypic (if it may be so expressed in the case of the germ cells) and as such, their further appearance is prevented by spermatogenesis itself.

Moreover, the negative result—even from the phenotypic point of view—in the majority of the breeding experiments after the X-raying of the germ cells, may be explained in the following way. The morphological modifications, found in irradiated testicles, are too rough to allow these monstrous spermatozoon to fertilise an egg; these spermatozoa are eliminated by way of simple resorption on the spot (Regaud). Those of them which are not altered morphologically and retain their mobility may also be eliminated by the loss of fertilising power (Bergonié and Tribondeau, 1904).

Only the least damaged spermatozoa can fertilise, and even these, although fertile, are very often unable to bring about a normal development of the embryo, as was first proved by Regaud and Dubreuil (1908) with rabbits. These authors mated males with irradiated testicles to normal females and examined the pregnant uteri before the end of a fortnight. They found in every case that the majority of the embryos were checked in their development at a very early stage, and that only a few, or sometimes none, were normal. Similar experiments with X-raying of ovaries were done by Lacassagne and Coutard (1923) and the result was the same: even ova which retained their capacity for being fertilised presented all kinds of abnormalities—interruption of development, absence of embedding, death of the embryo—which tended to suppress all abnormal foetuses.

Thus X-rayed germ cells of mammals may be supposed to present at least some of the chromatin modifications which have been found in plants and lower animals in a state of increased mutability. But a kind of natural selection taking place in the genitalia of higher animals (1) preserves posterity from any deleterious intervention in the germ plasm of its parents and (2) greatly diminishes the chance of survival of artificial mutants.

#### (b) *Mutation rate.*

In addition to visible modifications in the chromatin of irradiated germ cells, the rays are now supposed by many investigators to be able to produce real gene mutations. The new evidence obtained lately in *Drosophila* and in some plants and referred to in our introduction, have even permitted Muller to put this question: "Are natural mutations due to natural X-rays?" This question is very pertinent because the majority of the produced mutations are the same as those met with, although less frequently, in ordinary conditions without any treatment.

The rays quite evidently increase the rate of mutations. But as natural mutations, even in *Drosophila*, are very rare, the absolute increase in their number produced by the ray treatment is still very low. We have quoted the exact data in our introduction; but by way of illustration, a few instances may be recalled here: Muller obtained in the  $F_1$  generation 81 abnormal flies from about 2000 examined, and in the  $F_2$  generation, 88 lethals in 758 cultures; Hanson has found, in a more heavily treated series, 35 mutations (including the somatic abnormalities) in 1077 flies examined, and in a less heavily treated series—15 mutations in 2427 flies

examined; about the same proportions were also obtained in plants. It is hardly possible to increase substantially the rate of mutations by increasing the doses, because of the limit brought about by the sterilising effect of the rays. Moreover, in our experiments we did not get any abnormality in the progeny of heavily treated animals; only moderate doses yielded the abnormalities mentioned above.

If the rays, as a physical agent, were capable of producing mutations in any germ plasm, they would surely have done so much more frequently than has been found by those workers who have had the chance to obtain some positive results in this field. But there are also other observations: Mavor has obtained no conclusive evidence "that any individual gene or unit character has been altered, although during the course of experiments somewhat over one hundred thousand flies, developed from X-rayed eggs, have been studied"; Snyder did not notice any sign of abnormality of any kind in 1024 individuals, descendants of irradiated male rats; Bagg (in collaboration with MacDowell) could not reproduce the result of his original experiment, although several thousand animals were observed; negative results in the majority of our own experiments, etc.

All of these negative results are in accordance with the generally accepted difficulty of artificially changing the hereditary material of an animal. Quite recently, Hanson and Heys (1927) summing up their observations on the topic—"Do albino rats having ten generations of alcoholic ancestry inherit resistance to alcohol fumes?"—have been led to the conclusion, that acquired resistance is not inherited. Alcohol seems to act as a selective agent only, and produces no new heritable variations.

In a similar way, Zeleny (1928) on the basis of a general observation of the bar-eye stocks of *Drosophila* over a period of ten years, and a special experiment started in 1922, has concluded that it is highly improbable that there has been any significant inheritance of the temperature effect during the recorded period.

The dependence of gene mutation rate upon temperature was studied in *Drosophila* by Muller (1928) also and he has obtained but a slight increase in this rate with increase in temperature.

The stability of the sex ratio of a species has been scrutinised using alcohol with mice, and the majority of students during recent years (Crew, 1925; MacDowell, 1928) have obtained no modifications of the sex ratio in the progeny of treated parents.

On the other hand, without any treatment, Gowen (1928) has established a sporadically mutating stock of *Drosophila* which showed 34 sex intergrades and triploids on 1775 flies, whereas a normal stock of 15,785 flies showed but one triploid mutant and no sex intergrades.

In one of the above-mentioned works, Muller acknowledges that "instead of mutation proceeding at a fixed rate, as might have been supposed, it is exceedingly changeable in its frequency, and, in fact, variations of the order of 1000 per cent. can follow from unknown causes that invisibly differentiate experiments apparently similar, and involving only ordinary cultural conditions."

This variation of the natural mutability in different stocks of *Drosophila* as well

as the existence of other species which yield a certain percentage of unusual forms, mostly repeating themselves, reveals the heterogeneous composition of some species, the great majority of the individuals concerned being quite stable, and a small minority being in different degrees of predisposition to mutate. In a previous work we have advanced the hypothesis that the number of potential mutants, especially in species like *Drosophila melanogaster*, *Oenothera lamarckiana*, etc., may be much greater than the number of observed mutations. As the exact causes of spontaneously occurring mutations are so far completely obscure, this pre-existing predisposition of single individuals in a species may be supposed to involve their more ready response to the sometimes even unrecorded external influences, and so much more to such a powerful agent as are the penetrating rays.

The biological effect of the vibratory penetrating rays (X-rays and  $\gamma$ -rays) is dependent not only on the wave-length of the rays themselves, but also on the living object, the soil on which these rays have to act. All the studies made on normal tissues (muscles, testicles, lymphatic glands, etc.) as well as on neoplastic malignant growth, have shown the great difference which exists in the response of different living cells to the same doses. The most remarkable elective effect of the rays in cancer treatment is based on the more ready response to minor doses of the neoplastic cells, in comparison with the response of the environmental normal tissues. The different neoplastic tissues themselves also present a great variability in their radio-sensitivity in connection with their histological structure (radio-sensitivity of the squamous-celled epithelioma of the cervix uteri and non-radio-sensitivity of the glandular columnar-celled epithelioma of the body of the uterus), and the different reactions in individual cases, even with apparently the same histological form are well known to radio-therapeutists.

If this dependence of the biological effect of the rays on the object irradiated is a general law of radio-physiology, it is also to be taken into account in the evaluation of experimental results in the field of genetics.

The exceedingly meagre quantities of mutations obtained in comparison with the huge number of unchanged individuals in all experiments with the irradiation of germ cells, and the similarity of the majority of the mutations obtained with those occurring spontaneously, lead us to the conclusion *that the rays are unable to produce any new form*. The increased mutation rates, obtained lately in *Drosophila*, are probably an expression of the same elective action of the rays which has been so long studied on the normal and pathological tissues. Only in this case, not the cells of an organism, but the isolated organisms of a strain are in question. At any rate, the result is the same: the response is obtained only from those individuals which by their nature are predisposed to respond.

Nevertheless, the unquestionably established increase of mutation rate is a very valuable result of the recent intensive research in this field. It shows very evidently that the rays may be profitably utilised to detect the potential mutants in different species and to bring thereby to light such a condition of these individuals which, otherwise would probably remain without any manifestation.

Returning to our particular case, we may suppose that the male No. 10 was



a potential mutant to "waltzing" and the male No. 17 a potential mutant to "abnormal tail." The fact that the rays have produced but these two mutations, already known in this locality before, makes most probable the assumption, that the rays are not the cause of these mutations but only a revealer of a pre-existing latent condition.

As to the rôle played by "the natural X-rays" in the origin of "natural mutations"—after all that has been said, we cannot accept the hypothesis that they have any particular influence.

### (c) Hybridisation.

Another view concerning the method of evolution is that hybridisation is one of evolution's principal agencies. In the discussion of the theory of the origin of species through mutations, established by Hugo de Vries (1901) on the *Oenothera lamarckiana*, certain biologists, with Bateson (1909) at the head, advocated the hypothesis that this plant is nothing more than a complex hybrid which is giving the new forms by disjunction. Davis (1924) has even produced a plant resembling very much the *Oenothera lamarckiana*, by crossing *Oe. franciscana* with *Oe. biennis*.

More recently, Jeffrey and Hicks (1925) studying the reduction division in relation to mutation in plants and animals, have found the same lagging of chromosomes in meiotic division in *Drosophila* as was found by Rosenberg in a hybrid between the long leafed and the round leafed sundew (*Drosera longifolia* and *Drosera rotundifolia*).

Paralleled examination of the meiotic division in the grasshopper has shown a quite normal disposition of chromosomes. This allowed the authors to conclude that *Drosophila melanogaster* presents an example of a phenomenon which is extremely common on the plant side, namely the appearance of a natural hybrid.

Taking into account all these facts and also the suggestion of Lang that the anoure mouse may be a homozygote which has produced brachyures—intermediate hybrids—by crossing with normals, we have carried out about 1700 crossings and have obtained more than 4000 mice in our stock of "abnormal tail." Yet we have not arrived at establishing a strain of tailless mice which would breed true. The curtailed formula 2 : 1 of their disjunction concurs also in the inference that such a homozygous dominant anoure is lethal and does not exist in natural conditions.

This last statement makes impossible the origin of our animals with abnormal tail through a simple accidental crossing of two individuals—carriers of two allelomorph characters—because one of these individuals, homozygous tailless, does not exist. The only explanation of the origin of this abnormality is that it is a real mutation, due to a change in the hereditary substance, a change which has induced a heterozygous condition. The possibility of the transition of a homozygous germ plasm into a heterozygous has already been admitted by Fruwirth (1909) in his experiments with leguminous (*Pisum*, *Vicia*, *Lupinus*, *Faba*) and by Correns (1910)—for *Mirabilis jalapa*.

Discussing the question, why polyploidy is rarer in animals than in plants,

Muller (1925) admits, that "if only a small section of the chromatin were inactivated or removed from both members of a pair of homologous chromosomes, inviability should result." It may be supposed that in our case of tail abnormality, the rays have produced, at a point of diminished stability in one chromosome of a pair only, a kind of inactivation or elimination of a small portion of chromatin. This would explain the heterozygous condition in the mice of this strain, and their hereditary behaviour, as of hybrids. Eventual reunion of two such chromosomes would make the individual inviable. The alteration in one chromosome only of a pair was observed by Baur (1918-1922) on *Antirrhinum majus*.

The same immediate transition of a homozygous into a heterozygous condition might have taken place in connection with the origin of yellow mice and perhaps of other lethal characters, unknown in an homozygous state.

There is another argument against the hypothesis of the origin of our "abnormal tail" strain through hybridisation. It is generally accepted (Lang, Duboscq) that the character "short tail" is a dominant one, and, in fact, it has appeared in the descendants of the ♂ 2 since the first generation. Nevertheless, in the case of the ♂ 17, all the offspring of the  $F_1$  generation were long-tailed, and only a few of them had the tail a little bent. The short-tailed mice appeared no sooner than the  $F_2$  generation, and this shows that the ♂ 17 was not a hybrid in respect to "short tail," and that the abnormality originated in this case in some other way.

As regards our waltzing mutation, this might be translated, being recessive, as a latent hybrid state through many generations, reappearing, by inbreeding, in the  $F_2$  generation. But the extreme rarity of such a spontaneous reappearance and the absence of such a case in our recent control stock, incline us to the supposition that the rays in this case, too, have simply met a predisposed individual with a diminished resistance, a kind of unstable equilibrium, in a portion of a chromosome, corresponding to the labyrinth. The union of two such chromosomes in the  $F_2$  generation had no lethal effect, but resulted in the appearance of a homozygous recessive waltzing mouse which breeds true.

Comparing these two strains—short-tailed mice and waltzing mice—we see that their hereditary behaviour is quite different. The waltzers are always transferring the same character in crosses *inter se* and with normal mice. We have never seen anything else appear. Whereas in the strain with abnormal tail, many new forms have been observed which differ quite evidently from the initial mutation. Hence the continuous mutability is an inherent quality only of our second strain, and this is a phenomenon which cannot be explained by a simple hybridisation.

Such a condition of continuous mutability was first observed on the plant side (*Maize*, *Oenothera lamarckiana*, etc.) and described by De Vries under the name of "ever sporting varieties." Baur studied the same phenomenon on *Antirrhinum majus*, and Demerec on *Drosophila virilis* in which he described three such "mutable genes." The results of the experiments so far obtained, he explains by the assumption that the "mutable gene" is complex in structure. Elsewhere we named this complex formation in the hereditary substance, corresponding to the mutable tail in our mice, a "mutable factor," reserving the term "gene" for the real units, or

simple mendelian characters. For those atoms of heredity which constitute a "mutable factor" the name of "gene elements" may be maintained. The existence of such smaller units in our case reveals itself in the possibility of being able to reproduce many of the particular forms of abnormal tail in succeeding generations; our next problem is to extract at least some of them as pure as possible.

The assumption of such "mutable factors" accounts for the mutability in species like *Oenothera lamarckiana*, *Drosophila virilis*, etc. and also for a strain like that of our mice with abnormal tail, and shows that there are phenomena in the field of heredity which cannot be reduced to a simple hybridisation.

Though we can admit quite speculatively that, if in a mating of two individuals, belonging to two different races or species, one of these individuals is in a state of latent mutability ("pre-mutation" of Cuénot), such a mating can bring out this mutability and give rise to a mutating strain, producing forms which differ from the initial mates.

(d) Selection.

Holding as nearly as possible to the concrete results gained not only from the author's own investigations, but also from the above mentioned data of other students, we may be permitted to connect up these new facts with the general conception of the evolution of species. We fully realise that many of the questions before us cannot be answered with finality, but we believe that no one will deny that the time has come for some new departure in evaluation of accumulated observations.

The initial origin of species, like the origin of life in general, is up to the present beyond the scope of our scientific tools. All that we know deals only with the evidence regarding either continuous transformations of the forms of life through natural selection, or discontinuous, by means of mutations.

The true nature of mutations, together with their underlying causes, is still by no means understood. Bateson's general conclusion is summed up in the statement that "the discontinuity of which species is an expression, has its origin not in the environment, nor in any phenomenon of adaptation but in the intrinsic nature of organisms themselves manifested in the original discontinuity of variation." And further: "the discontinuity of species results from the discontinuity of variation."

This statement gives us a line of approach in analysis of the subject and emphasises the rôle of the "intrinsic nature of organisms" in the origin of new forms. But from our point of view, it need be limited only to a certain amount of exceptional organisms.

In effect, the selection experiments of pure lines, as, for instance, those with beans, have shown the extreme stability of a genotype and the impossibility producing a new form with sensibly differing weight by pure selection (Johannsen, 1903). The small phenotypical modifications dependent mostly on environmental conditions, which appear continuous in a population, were found to form regular curves, and it is exactly these curves that prove to be characteristic and constant for every genotype.

Selection gave a positive result to De Vries in the transformation of an ordinary

*Chrysanthemum segetum* into a *Chr. segetum grandiflorum plenum*, because he had started his selection with a plant already deviated from the primitive form. The selection only fixed and brought to full development the change which had occurred spontaneously before.

The same process may be supposed to have taken place in the development of living material in general.

At any moment of their history, the existing species are stable, and only this stability of hereditary substance can explain why there are still very ancient species on the earth, such as some of the algae, insects, etc. The "intrinsic nature of organisms" composing all these species manifests itself in such a way that they transmit their genotypical structure unchanged to their descendants. The environmental conditions, the struggle for existence, use or disuse, the action of all kinds of physical, chemical, mechanical and other agents can only produce individual phenotypical changes which are not transmitted by heredity (Bonnier, 1895, experiments on *Teucrium*).

It can hardly be expected that a species, as a whole, could enter into a period of mutability—a hypothesis which was advanced by Dr Vries on behalf of *Oenothera lamarckiana*. Isolated cases of mutations in different species were known long ago, and many of them have been collected by Darwin, Korschinsky, etc. and others have been recorded from time to time by different authors. What the experiments described above seem to establish is that there may be isolated individuals with latent instability of their hereditary material, even in species which seem to be quite stable, as was the case with our mice.

Only these particular organisms will correspond to Bateson's statement, and at least a tendency to variation will be their "intrinsic nature:" Under ordinary conditions very many of them do not perhaps manifest their particular condition: sometimes they die early because of the lethal character of the mutation, as hereditary anaemia in mice (De Aberle, 1927), or "hairless" a new recessive lethal character in cattle (Mohr and Wriedt, 1928), sometimes they are not fertile, sometimes the new character is a recessive one, and it does not appear in the first generation after a mating with a normal mate; it is necessary to wait for a random crossing of two hybrids to obtain the new character in full evidence.

The positive results of some attempts to change the hereditary behaviour of some living organism artificially, may depend on the fact that precisely these unstable individuals have been occasionally detected and their mutability brought into activity by means of an external agent. It may be further supposed that these individuals, unbalanced from an hereditary point of view, and therefore more sensitive to all kinds of agencies, are exactly those which respond to the changes in environment, and originate new characters more readily.

The nature of these new characters does not in general depend on the external agent but on the predisposition which existed in the hereditary material beforehand; therefore, there is no connection between these two factors, and new traits, as "short tail" or "waltzing" in our case, seem to occur by chance.

Morgan, Sturtevant and Bridges (1928) have attempted to find out whether

an extensive injury can have any influence in increasing the percentage of mutations in *Drosophila melanogaster*. The eyes of newly hatched females were touched with the tip of a hot needle. The result was that more mutants were found in the offspring of the burnt-eyed flies than in the extensive controls. But the mutant characters appeared in different parts of the body and not more frequently in the eyes than elsewhere.

The sensitivity of the mutating individuals to environmental influences is well established by Morgan on *Drosophila*, and it will be sufficiently illustrated by the following instances.

(1) There exists a mutant race of *Drosophila* in which the pigment bands on the abdomen are extremely irregular, or even absent altogether, but only when the flies are reared in good food conditions. When the external conditions become less favourable, the pigment bands tend to become more and more normal, until at last all the flies that emerge are indistinguishable from normal ones. If abnormal abdomen is crossed with normal, the  $F_2$  generation consists of 3 abnormal to 1 normal in good food conditions; in poor conditions there may be no flies with abnormal segments.

(2) There is a race of *Drosophila* with one, two or even all its legs doubled. This race differs from the normal by a single gene; the character appears only when the flies are reared in an ice-chest, then good mendelian ratios are obtained; if they are reared at room temperature, no flies result with more than six good legs.

In these two cases the natural susceptibility to changes occurring in environmental conditions pre-existed in these animals, and that is why the external agencies could produce a visible effect. It was the same, I believe, with the mutations in our mice in respect to the rays. The laboratory selection permitted us to save these mutations and to bring one of them—abnormal tail—to further development but not to produce them. The rôle of selection in nature is rather to limit the variability, and the chance of survival of the majority of natural mutations is very small, especially for the homozygous recessive as was calculated by Fisher (1928). The heterozygous dominants are in a better state as they reappear in crosses with the normals from the  $F_1$  generation in 50 per cent. of the offspring.

But the phenomenon of dominance itself does not always appear quite definitely (Baur); we may refer to our case with the ♂ 17, where the dominant character of the "short tail" did not manifest itself in the  $F_1$  generation. Punnett states also in his *Heredity in Poultry* that several mutants among comb characters, e.g. both "pea" and "duplex," show very variable degrees of dominance in different breeds. Fisher has even advanced a "theory of the evolution of dominance by selection."

Returning to the question of the evolution of living material, we must remember the words of Morgan: "If we suppose that new mutations and 'definitely' inherited variations suddenly appear, some of which will find an environment to which they are more or less well fitted, we can see how evolution may have gone on without assuming that new species have been formed through a process of competition. Nature's supreme test is survival. She makes new forms to bring them to this test through mutation, and does not remodel old forms

through a process of individual selection." A creative act of nature precedes selection. What natural selection does is only this, that it determines the survival of those new features which have pre-eminently an adaptative character, and this explains the adaptative character of species themselves.

There still remains a further question before us, namely the reproductive isolation of species and the impossibility of intercrosses between them, whereas the new mutants conserve in general their capacity for being crossed with original forms. To illustrate how the question of species crossing presents itself now in scientific opinion, we may refer to the following recent observations. In a short critical note in the *American Naturalist* Castle (1925) exposes a thoroughly critical experimental investigation of the question made by two Japanese zoologists, J. Yamane and T. Egashira, *Dobutzu-Gaku Zasshi* (Japanese), 1924, vol. 36, No. 430. It deals with experiments, attempted both by natural and artificial fecundation, in crossing the domestic rabbit with the Japanese subspecies of the European hare. The authors conclude that in their experience it is impossible to get hares to mate naturally with rabbits, and even if this were to occur under exceptional circumstances, no hybrid offspring would result because of the evident inability of the rabbit egg to be fertilised by hare sperm. Castle finishes this exposition by the words: "We may accordingly relegate the hare-rabbit to the limbo of zoological myths."

Only slightly more encouraging results were obtained by Chittenden (1928) on plants. In the *vernales* section of the genus *Primula*, all the species examined had the same chromosome number ( $n = 11$ ) and all when intercrossed gave very fertile hybrids.

In *Godetia* species examined presented two groups: *A*, all with seven chromosomes haploid, and *B*, with different numbers of chromosomes. Intercrosses in each group gave completely sterile hybrids; species from one group did not intercross in any way with species from another group.

Six species were examined in *Nemophila* four of which are closely allied. All these species ( $n = 9$ ) are completely intersterile. In *Phacelia* four species were examined ( $n = 11$ ), and only two, *P. Parryi* and *P. Whitlavia* gave a fully fertile hybrid. The author remarks on this occasion that "it is possible that, in spite of their marked vegetative differences, they are not really distinct species."

The origin of this specific isolation is perhaps one of the most obscure points in the whole problem of species. It needs, I believe, a very prolonged and extensive study of mutating strains, such as our mice with abnormal tail, mutating strains in *Drosophila*, etc., with a view to extracting mutating individuals which would not intercross with original forms.

#### (e) Summary.

1. The irradiation of the testicles in mice, with all kinds of doses till that giving complete sterilisation, did not produce in the majority of cases (about 3000 descendants) any hereditary effect.

2. Two mutations only (on 35 irradiated breeders) were obtained: first, a waltzing mouse, breeding true as mendelian recessive, and second, a short-tailed



mouse, so far only known in the hybrid state, but mutating continuously and giving different new forms of tail (anoure, filiforme tail, kinky brachyure, helicoid type, interruption of the skeleton, etc.).

3. These two mutations—"waltzing" and "short tail"—had already been observed in this locality by previous investigators, and besides, one male of our own stock presented a small tail abnormality, which passed unperceived before the irradiation and which proved afterwards to be hereditary. The mutations obtained must not, therefore, be considered as new ones produced by the rays. It was concluded that the rays only revealed a pre-existing latent state which otherwise would have remained undetected.

4. New evidence supporting the last assumption is the non-appearance of waltzing or of any tail mutation in a new stock of more than three thousand control mice—descendants from a few normal parents and reared by inbreeding.

5. In the light of these results, the living hereditary material, as represented by different species, may be considered to be quite stable and incapable of being changed in its genetic structure, by any kind of external agency. This accounts for the existence of very ancient species on the earth, for the extreme rarity of the natural mutants, and for the negative results of a great number of attempts to change hereditary behaviour of animals and plants by artificial means.

6. The variability of species and the recorded cases of positive results in attempts to produce a new form by means of different artificial procedures, may be accounted for by the assumption that there exists in different species a certain number of scattered single individuals—potential mutants—whose hereditary material conceals different degrees of instability.

7. This instability probably remains very often unperceived; sometimes it may manifest itself, as well in laboratory conditions as in the state of nature, through spontaneous mutations—source of variation. But the rate of these mutations is in general a very low one, even in *Drosophila*. This rate may be greatly increased, and the concealed mutability of some apparently normal individuals brought to light, by means of different external agents, especially by irradiating the gonads of the animals used for breeding.

8. The utilisation of penetrating rays seems to be a good method, as they appear to have an elective action on the potential mutants, as was shown by our own findings and by all the recent successful experiments on *Drosophila*. Yet the rays can hardly be looked upon as a real cause of mutations, and, therefore, the term of "producing" mutations were better abandoned as a misleading one.

9. Hybridisation, natural selection, etc., cannot create new forms. An independent act of nature, creating at least a predisposition to change, precedes all other agencies, though it may not always be at once evident.

10. The rôle of natural selection, as a survival in competitive conditions, is to fix a few of the new forms which are better adapted to a given environment, and to eliminate the majority of them. Hence natural selection cannot be considered as an agent favouring the variability of species on the earth, it is rather an agent limiting this variability.

11. Artificial selection made for purposes of domestication and under laboratory conditions tends, on the contrary, to conserve those mutations appearing spontaneously, or revealed by artificial means, which would perish under nature conditions.

12. This hypothesis of *stable species with single changeable individuals* amongst them, which are the source of new forms on the earth, would settle numerous experimental controversies. Moreover, it is in full accord with the established impossibility of the inheritance of somatic fluctuations resulting from the adaptation of the individuals to environmental conditions.

13. The present hypothesis conceives the building of organic life resulting from the process of evolution as based on three pillars: (1) stability of existing species as the expression of the conservative principle of life, (2) variability of single individuals as the manifestation of the creative power of nature, and (3) natural selection as the casting away of products which appear less fitted for the struggle for existence under given conditions, and thus determining that the species are adapted to the environment. The struggle for existence plays a special part in phenotypic perfection of individuals.

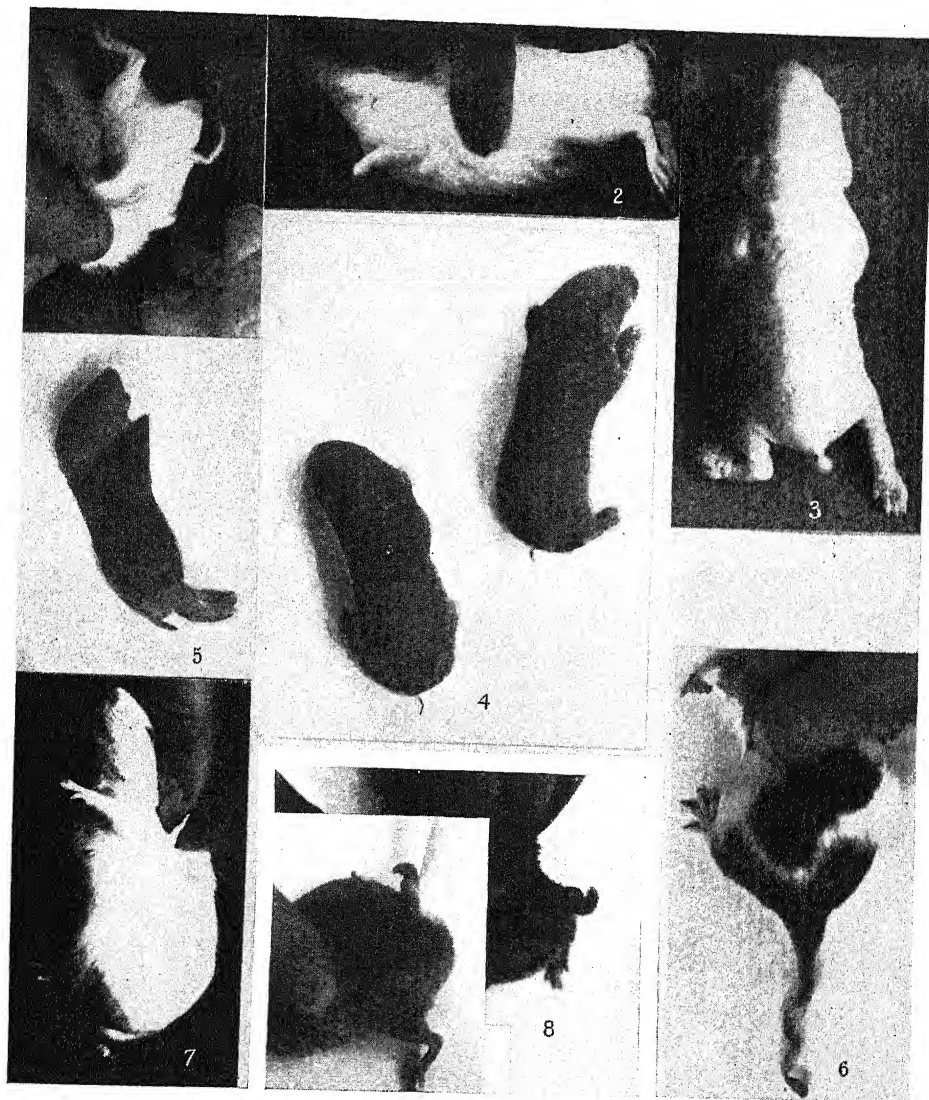
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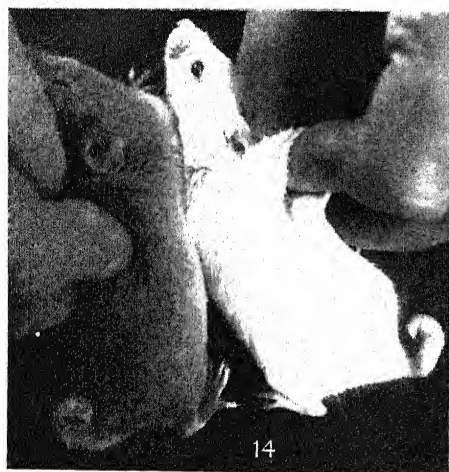
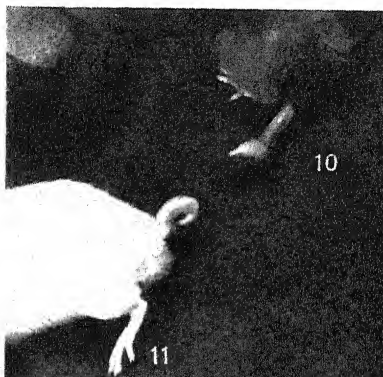
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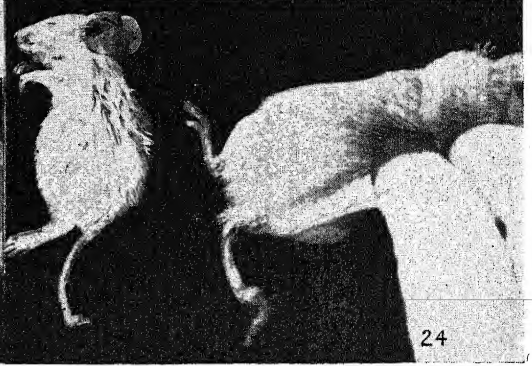
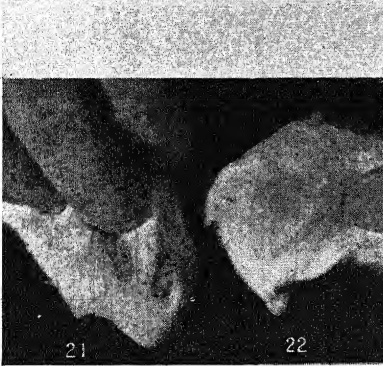
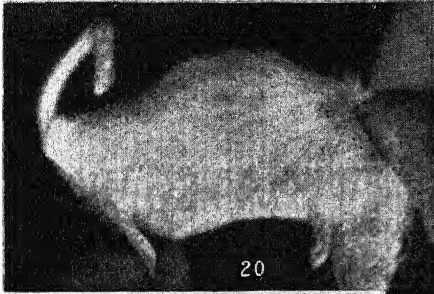
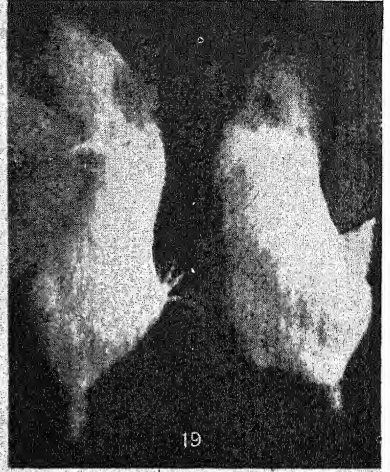
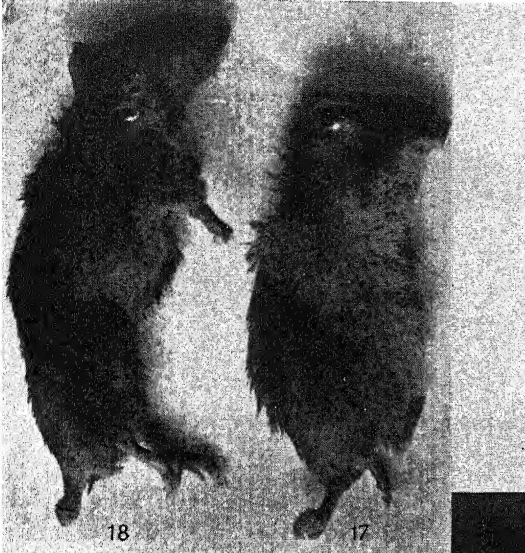
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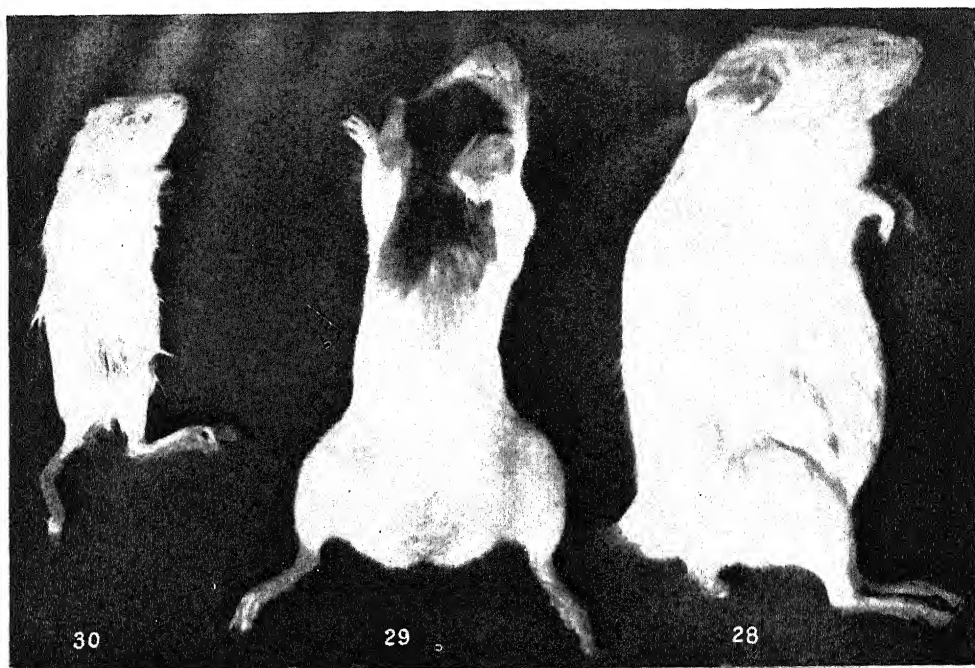
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# OXIDATION MECHANISMS IN ANIMAL TISSUES

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(Received July 27, 1929.)

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## I. INTRODUCTION.

THE necessity of a continuous supply of oxygen to the animal organism, in order to bring about the oxidation of the organic fuel materials derived from the foodstuffs with a consequent liberation of energy essential for life, has been realised since the work of Lavoisier in 1770. Until recently, however, the study of the mechanisms by which the reaction between oxygen and the organic materials takes place in the tissues of the animal body was unduly neglected. A good deal of attention had been given in the meantime to the absorption of oxygen and food products into the blood stream and their carriage to the tissues, but very little real progress was made in knowledge of the means by which they are caused to react together in the tissues to supply the energy necessary for life. It is practically only during the last ten years that most of the progress in this important problem has been made. Much attention is however being given to it at the present time, and very recent work has done much to elucidate and co-ordinate what was a rather complex and obscure subject. This article is an attempt at a survey of the present position, with particular reference to the bearing of recent work.

Since the subject of tissue oxidations deals with the reactions taking place between molecular oxygen and combustible organic metabolites, it is related on the one hand to tissue respiration (the absorption of oxygen by the tissues), and on the other to intermediary metabolism (or rather that branch dealt with in Dakin's (1922) monograph). These are in fact merely the same process viewed from two different aspects.

Now the organic substances oxidised in the tissues are practically all stable to oxygen apart from the tissues. Molecular oxygen is in fact only a mild oxidising agent, and the organic metabolites are not as a rule reducing agents. They can be left in contact with air for indefinite periods without undergoing oxidation. Yet in the tissues they readily become oxidised by oxygen. This, the fundamental phenomenon which constitutes our problem, is due to the presence in the tissue cells of

a number of enzyme catalysts of oxidation, which are together responsible for tissue respiration.

These enzymes are known by the general name of "oxidases."<sup>1</sup> A number of oxidases have been extracted from the tissues and studied separately.

The efficiency of these enzymes is quite remarkable. For instance, succinic acid and the purine base hypoxanthine are both difficult to oxidise by ordinary chemical means—they will withstand boiling with strong nitric acid. Yet if they are added to, say, an aqueous extract of liver they become readily oxidisable by even the mildest oxidising agents, including oxygen, owing to the action of the enzymes succinoxidase and xanthine oxidase present in the extract.

It is now quite clear that the oxidases present in the tissues are not all of the same type and act quite in different ways. They can be classified into a few main groups according to their mode of action, and in this article we shall consider the action of the various types, the relation and co-ordination of the different types with one another, and their relation to tissue respiration as a whole.

Before, however, dealing with oxidases themselves it will be necessary to devote some space to a preliminary consideration of oxidation and reduction reactions generally, and to a brief mention of the various theories of the mechanism of such reactions.

We may conveniently take first points relating to the nature of the oxidation-reduction process itself, and in the second place points relating to the possible modes of catalysis of such reactions.

#### *The oxidation-reduction process.*

We may take as a starting-point two elementary but fundamental considerations.

1. The oxidation of an organic compound may involve either a gain of oxygen atoms or a loss of hydrogen atoms. The conversion of an alcohol into an aldehyde is just as truly an oxidation as the conversion of the aldehyde into its acid, although the first consists in the loss of two hydrogen atoms and the second in the gain of an oxygen atom. Similarly reduction, being the converse of oxidation, may involve either a gain of hydrogen atoms or a loss of oxygen atoms.

2. The oxidation of any substance involves the simultaneous reduction of another substance, and *vice versa*. This follows from the nature of oxidation and reduction. Two reactants therefore always take part in any such reaction, namely the substance which becomes oxidised (for which the general symbol *A* will frequently be used here) and the substance which oxidises it (which we shall call *B*); or otherwise expressed, the substance which becomes reduced (*B*) and the substance which reduces it (*A*). (Neglect of this fact has not infrequently led to some confusion of thought; as, for instance, in the statement sometimes made that some regions of the cell are places where oxidations occur and others are places of reduction—a statement which is meaningless as it stands.) In the absence of any settled nomenclature *A* may be referred to simply as the reducer and *B* as the oxidiser in

<sup>1</sup> The term "oxidase" has occasionally been used in the past with various restricted meanings, but it is preferable to keep it as a general term.



the reaction. In the tissues, of course,  $A$  will represent the various metabolites derived from the food materials, and  $B$ , ultimately at any rate, is oxygen itself.

From facts 1 and 2 it follows that, since  $A$  can either lose hydrogen atoms or gain oxygen atoms, and  $B$  can either gain hydrogen atoms or lose oxygen atoms, four types of reaction are possible. These may be represented as follows:

- (a)  $AH_2 + B = A + BH_2$ .
- (b)  $A + BO = AO + B$ .
- (c)  $A + H_2O + B = AO + BH_2$ .
- (d)  $AH_2 + BO = A + H_2O + B$ .

In types (a) and (d) the reducer is a "hydrogen donator," in (b) and (c) an "oxygen acceptor"; in types (a) and (c) the oxidiser is a "hydrogen acceptor," in (b) and (d) an "oxygen donator."

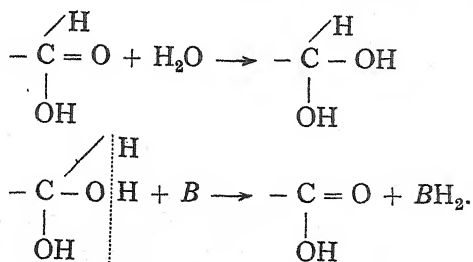
This scheme is, of course, merely a convenient general classification of the reactions according to the final result of the process. It assumes nothing about the actual mechanism of the reactions. There is no evidence of any fundamental difference in mechanism between the various types; they may, and probably do, take place by the same mechanism. It is worth while pointing this out, because there has been a tendency for the supporters of the various theories of the mechanism of oxidation reactions to select that type which best accords with their particular theory, and to stress the importance of the reactions belonging to that type.

The net result of a reaction of type (a) is the transfer of hydrogen atoms from one molecule to the other, type (b) results in the transfer of an oxygen atom, type (c) is the so-called "hydrolytic oxidation-reduction," and type (d) is the converse of type (c).

We must now consider briefly the current theories as to the mechanism of such reactions. It does not seem possible on the evidence at present available to decide definitely which is to be regarded as the correct one, and of course it is by no means sure that all oxidations take place by the same mechanism. It appears to be possible to explain the facts on any mechanism, and it is therefore perhaps not of the greatest importance to give a definite decision. (This only applies, of course, to the theories of the reaction process itself, and not to the theories of oxidase action discussed later.) We shall not attempt it here. At present it seems that that view should be adopted which gives the simplest interpretation of the facts.

1. *Wieland's theory.* (For a fuller account of this theory see Wieland, 1922). According to this view, oxidation-reduction reactions actually take place by a direct transfer of hydrogen atoms from one molecule to the other. Oxidations are thus essentially dehydrogenations. The theory, which has come into considerable prominence during the last few years, is particularly adapted to explain reactions of type (a). One of the main reasons why the theory is so useful is that practically all oxidations of organic compounds actually produce a loss of hydrogen atoms. Only in a very few cases, of which the oxidation of aldehydes is the most important case, does the oxidation result in a gain in oxygen atoms. Wieland's method is thus the simplest way of expressing the great majority of organic oxidations.

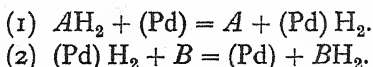
In those few cases in which there is a gain in oxygen atoms Wieland assumes a preliminary hydrate formation. Thus in the case of aldehydes:



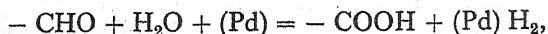
This undoubtedly gives a very clear picture of the reaction, and in support of it Wieland quotes the fact that chloral itself dissolved in benzene cannot reduce silver oxide, whereas chloral hydrate in benzene solution is able to do so.

The necessity of assuming a hydrate formation in such cases cannot be regarded as an objection of any weight against the theory. The few cases other than aldehyde are mostly reactions in which the molecules are activated by a catalyst, and, as we shall see later, it is quite possible that molecules activated in this way may form hydrates even when the molecule in the unactivated state does not.

The fundamental observations on which Wieland largely based his theory are briefly as follows. Platinum or palladium "black" (the metal in a finely divided condition) has the well-known property of absorbing considerable quantities of hydrogen as a kind of loosely combined hydride. By making use of palladium black as an "intermediate hydrogen acceptor" Wieland was able to resolve the oxidation-reduction reaction into two successive stages, which for a reaction of type (a) may be represented thus:



He found that on bringing aqueous solutions of many oxidisable organic substances into contact with palladium black (in the absence of oxygen) the substances became oxidised and the palladium became charged with hydrogen (stage 1). On removing the hydrogenated palladium and placing it in contact with solutions of reducible substances, these then became reduced (stage 2), or alternatively, on heating the palladium, molecular hydrogen was given off. As reducible substances (B) compounds such as quinone, reducible dyes such as indigo and methylene blue, and molecular oxygen itself could be used, among others. Aldehydes were among the substances found to be oxidised by such a system. On adding palladium black to an aldehyde solution the aldehyde becomes oxidised to its acid, while the palladium becomes charged with hydrogen as before:



so that it is clear that the hydrogen in this case comes from water molecules taking part in the reaction either by hydrate formation, as assumed by Wieland, or in some other way.

A solution of glucose, when brought into contact with palladium black, becomes oxidised in the complete absence of oxygen, giving off  $\text{CO}_2$  and charging the metal with hydrogen, which can be transferred subsequently to molecular oxygen. Thus the oxidation of glucose, which was formerly thought to be brought about by an active form of oxygen (see later) may in fact be a process of dehydrogenation. It must be noted, however, that the palladium in this case, in which the substance undergoing oxidation is not "active," is playing a dual rôle. It is not acting merely as a passive hydrogen acceptor, but also as a catalyst, activating the molecules in contact with its surface. The question of activation is considered later.

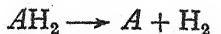
This work of Wieland, which was first published in 1912, had a considerable influence on the tendency of thought on the subject, and suggested the theory of hydrogen transfer, which is one of the more important theories at the present time. We shall see, however, that these facts are capable of explanation on other lines, and cannot therefore be regarded as proof of the theory.

We may picture the transfer of hydrogen atoms from one molecule to the other conveniently, if somewhat crudely, on the following lines. The molecular structure of the reducer ( $\text{AH}_2$ ) is such that the particular hydrogen atoms in question are only attached to the molecule by comparatively weak attractive forces. (Wieland calls such loosely attached hydrogen atoms "active hydrogen," whether they are attached to an organic molecule or a metal surface, etc. The weaker the attractive force the closer the approximation to atomic hydrogen.) The molecules of the oxidiser ( $B$ ), in virtue of their structure, have a field of attraction for hydrogen atoms, which, if the reaction is to proceed, will be stronger than that of  $A$ . As the molecule of  $B$  approaches that of  $\text{AH}_2$  closely, a point will come where the attraction of  $B$  on the hydrogen atoms exceeds that of  $A$ ; a "rearrangement of bonds" will take place, and the atoms become attached to  $B$ .

This method of picturing the process, in which any assumption as to the nature of chemical forces has been purposely avoided, prevents certain rather common misconceptions of the assumptions involved, as, for instance, that the hydrogen passes as a hydrogen molecule or as free hydrogen atoms. The atoms never become free, since they never escape from the field of one or the other molecule, neither do they join up to form a hydrogen molecule. The convenient common method of expressing one side of the reaction only



is liable to suggest that the reducer molecules undergo an actual dissociation giving off hydrogen atoms into the solution, and should only be used with the clear understanding that it represents an imaginary process. The common practice of writing

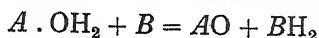


or



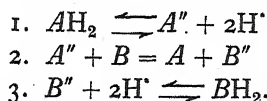
should certainly be avoided. Neither hydrogen molecules nor hydrogen ions have reducing properties.

Of course any of the four reaction-types can be written in terms of Wieland's mechanism by assuming a hydrate formation. Type (c) for instance becomes



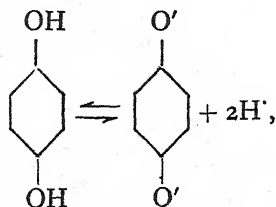
2. *The electron transfer theory.* This theory, whose biological application is more recent, regards the essential oxidation-reduction process as a transfer of electrons from the reducer to the oxidiser. It has the merit of being in line with current tendencies in physical chemistry. But while it is convenient for describing the oxidation and reduction of ionised systems, such as inorganic salts (e.g.  $\text{Fe}^{++} \rightarrow \text{Fe}^{+++} + \oplus$ ), it is perhaps less convenient for expressing the oxidation of organic substances, as their ionisations have to be taken into consideration.

On this theory a reaction of type (a) would take place as follows:



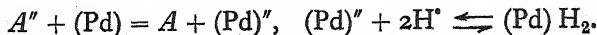
The first stage is simply the ionisation of the reducer as an acid, giving off two hydrogen ions. The anion of the reducer then reduces *B* by transferring to it a so-called "electron pair," this constituting the actual oxidation-reduction process. The anion *B''* then combines with two hydrogen ions. The net result is the passage of two electrons and two hydrogen ions from *A* to *B*, and it will be seen that the essential difference between this mechanism and the last is that here the electrons and hydrogen ions pass separately, whereas Wieland supposes them to pass together in the form of hydrogen atoms—possibly not a very fundamental difference.

In a large number of cases the active hydrogen atoms are more or less acidic, as for instance in hydroquinone,



but in a few cases they exhibit no acidic properties, and in such cases the theory in this form becomes somewhat artificial. It is however widely adopted.

Wieland's palladium experiments are explained equally well on this view as follows:



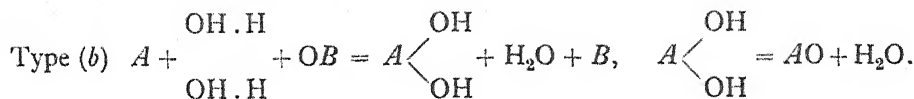
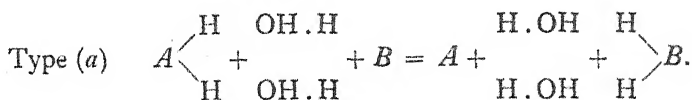
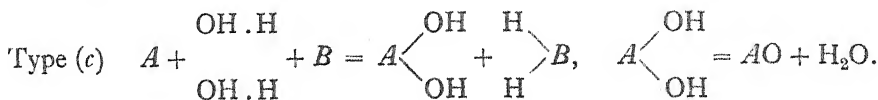
In the second stage the palladium is acting somewhat like a hydrogen electrode (see Dixon, 1927, 1).

3. *The hydrolysis theory.* This theory, which was developed chiefly by Traube and Bach, was suggested by a consideration of those reactions belonging to type (c). It assumes that the reaction takes place by the addition of the elements of water to oxidiser and reducer, the hydroxyl groups going to the latter and the hydrogen

to the former. In the original non-ionic form the theory supposed the addition of electrically neutral groups, but there is a later form in which hydrogen and hydroxyl ions are assumed to be taken up.

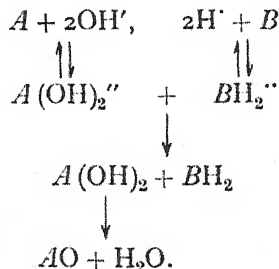
(1) *Non-ionic.*

The following equations will make the process clear:



(2) *Ionic.*

Type (c)



The ions of water are first taken up as indicated in the pair of reactions in the first two lines. The molecules thus formed then react together by electron transfer to give the products in the third line. It will be seen that this is merely a variant of the electron transfer theory, the ions being taken up before the transfer of electrons instead of after. On structural grounds the other form seems preferable.

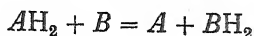
4. *The oxygen atom transfer theory.* This, which would correspond to reactions of type (b), is the logical counterpart of Wieland's theory. Owing, however, to the fact that most organic oxidations involve a loss of hydrogen atoms, it is inconvenient and not much advocated.

It will now be clear that practically any reaction can be written in terms of any mechanism by making simple assumptions. All the mechanisms ultimately come to much the same thing, and, in the absence of any clear experimental evidence as to which is the actual one, that view should be adopted which expresses most simply the processes being considered. Undoubtedly for our purpose the Wieland view is the simplest, and it will be adopted here, but this must not be regarded as a denial of the possibility that the reactions take place by electron transfer.

*Catalysis and activation*

In order that a reaction may proceed, two conditions must be fulfilled.

1. The energy conditions must be right. In a reaction



the tendency for *B* to become *BH*<sub>2</sub> must be greater than that for *A* to become *AH*<sub>2</sub>. In other words, the affinity of *B* for hydrogen must be greater than that of *A*; or *B* must be a "strong" enough oxidiser to oxidise *AH*<sub>2</sub>. This is a condition relating to the equilibrium point of the system, and its study belongs mainly to the domain of "oxidation-reduction potential." No attempt will be made to discuss this subject here; Michaelis's monograph (1929) should be consulted.

2. Both reactants must be "active." The word "active" will not generally be used here in the strict physico-chemical sense, but somewhat more loosely. There is a large class of oxidisers and reducers, including the ordinary oxidising and reducing agents, many dyestuffs, etc., which react together, provided condition 1 is fulfilled, without requiring the presence of a catalyst. It is convenient to speak of these substances as "active." On the other hand, there is a considerable number of substances, including most of those concerned in biological systems, which cannot react even with active oxidisers or reducers unless an appropriate catalyst is present. These are said to require activation by the catalyst.

According to Wieland, an active reducer is one whose molecule contains active hydrogen atoms. In accordance with this view the activation of a molecule by the catalyst involves such a change in the configuration of the molecule, produced by its adsorption on the catalyst, that the attraction of the molecule for certain of its hydrogen atoms becomes weakened, so that these atoms become active. This may be termed Wieland's theory of activation, to distinguish it from his theory of hydrogen transfer. The process is sometimes referred to as "hydrogen activation," since previously inactive hydrogen atoms become active, but it is probably preferable to think of the molecule itself as becoming activated.

Wieland's activation theory obviously deals only with the activation of the reducer. But it is not difficult to imagine that the activation of the oxidiser may take place on similar lines. We may suppose that the molecule becomes altered by its adsorption on the catalyst so that it develops a field of attraction for hydrogen atoms. But obviously no "hydrogen activation" is involved in this case.

It should be made clear that the molecules only remain activated as long as they are actually at the catalyst surface. Activation is not a permanent change in the molecule.

An electronic theory of activation has been developed by Quastel (1926), and applied to bacterial oxidations. This theory regards the essential activation process as being an electrical polarization of the molecule induced by intense local electric fields at the surface of the catalyst. As it is understood that this theory will be dealt with in an article by Dr Quastel, which is shortly to appear in this journal, it will not be treated here.



We can now make a classification of oxidation reactions on the basis of the type of catalytic mechanism involved, since either the oxidiser or the reducer or both may require activation. This classification, which is of course quite independent of the previous one, is of more fundamental importance.

Reducer (A)	Oxidiser (B)
1. Active	Active
2. Activated by catalyst	Active
3. Active	Activated by catalyst
4. Activated by catalyst	Activated by catalyst

In this scheme type 1 does not involve catalysis, and such reactions will proceed, if condition 1 above is fulfilled, in the absence of any catalyst. The other types are catalysed reactions. Each of these classes can of course include reactions of any of the types (a), (b), (c), (d).

A further catalytic mechanism, which cannot be regarded as activation of either reactant, is represented by the intermediate "hydrogen carrier." It occasionally happens that a reducer and an oxidiser, both active (or activated) as judged by their power of reacting with other oxidisers and reducers, are unable to react with one another for some unknown reason, but will do so if a third substance is added. For instance the amino acid cysteine cannot reduce silver salts. It can however reduce quinone to hydroquinone, and hydroquinone can reduce silver salts to metallic silver. The addition of a little quinone to cysteine enables it to reduce silver. Such a mechanism can of course occur in reactions of any of the types 1-4.

Catalytic oxidase systems corresponding to each of these types occur in the living cell, and this classification will be used to some extent as a plan in the treatment which follows.

### *Methods of studying biological oxidations.*

It may be helpful to give here a classified list of the types of experimental methods available.

#### 1. *Direct estimation.*

(a) Of reducer. Estimation of reducer (metabolite)  
Estimation of oxidation-product.

(b) Of oxidiser ( $O_2$ ). Measurement of  $O_2$  uptake, e.g. in Barcroft apparatus.

#### 2. *Substitution methods.*

(a) For reducer. "Peroxidase reagents." Guaiacum, benzidine, indophenol reagent, polyphenols, etc.

(b) For  $O_2$ . Methylene blue, nitrobenzene, etc.

We can obviously follow the progress of the reaction by determining the amount of either reactant undergoing change, that is to say, we can follow the reaction by estimations of either the substance oxidised (or its oxidation product) or the oxygen used in its oxidation. The principle of the much-used substitution methods is as follows. When we are studying the activation of one reactant only, and the other reactant is active, we can substitute for the active reactant another more convenient

active reactant. For instance, reactions of type 2 can be followed by method 2 *b*. In following the oxidation of hypoxanthine by the xanthine oxidase, which involves the activation of the hypoxanthine, methylene blue, an active oxidiser, can be substituted for oxygen, and the reaction followed by the colour change occurring as the dye becomes reduced. This is a more convenient method than the rather cumbrous one of oxygen uptake measurement. Similarly, reactions of type 3 can be followed by method 2 *a*. The so-called peroxidase reagents are mostly substances which give an intense colour when they become oxidised. Again, in reactions of type 4 the two activations can be investigated separately by employing substitution methods. Many examples of these methods will be given later.

With these introductory remarks we can now pass to a consideration of the oxidases themselves.

## II. THE DEHYDRASES.

The earlier workers on biological oxidations were impressed by the comparative inertness of molecular oxygen as an oxidiser of organic substances, and sought to explain all tissue oxidations by assuming the formation of various active forms of oxygen.<sup>1</sup>

Schönbein about 1870, influenced by his discovery of ozone, thought that the oxygen was converted in the tissues into ozone, which was responsible for the actual oxidations. This view has of course long been superseded. Ozone is incapable of oxidising many metabolites.

Bach in 1903 suggested that an organic peroxide was formed thus  $X + O_2 = XO_2$  where  $X$  is some organic substance in the tissues. Although the evidence for the formation of a true organic peroxide was not very strong, the idea was dominant for many years and is still of importance. It will be further discussed in a later section.

The work of Wieland (1912 onwards) drew attention to the importance of the activation of the metabolites to be oxidised, which had largely been lost sight of, and showed, as has been mentioned, that oxidations which had been thought to be due to some active form of oxygen could take place in the entire absence of oxygen. The application of this view to oxidations in animal tissues was greatly advanced by the work of Thunberg (1920). He demonstrated, by means of the methylene blue technique, that animal tissues contain oxidases capable of activating a considerable number of organic substances. These important enzymes, now generally known as "dehydrases,"<sup>2</sup> are systems concerned with reactions of type 2 in the scheme given above. A dehydrase then is an oxidase which acts by direct activation of the organic substance to be oxidised, rather than of molecular oxygen.

<sup>1</sup> An excellent account of the older work on oxidases is given by Kastle (1910). See also the review by Batelli and Stern (1912, 2), which contains a full bibliography. A very complete survey of the more recent literature on the subject will be found in Oppenheimer's book *Die Fermente* (1926), pp. 1213-1414, 1706-1871.

<sup>2</sup> The present confusion of nomenclature is illustrated by the fact that the following synonymous terms are actually in use at the present time to denote this class of oxidase: dehydrase, dehydrogenase, reductase, anaerobic oxidase, hydrogen transportase, oxidoreductase, redoxase, perhydridase, "water-splitting" enzyme, and some others. Of these "reductase" seems the most free from objection, since the enzyme acts upon the reducer. "Dehydrase" is however the term in most common use, and it will be retained here.

The methylene blue technique developed by Thunberg has been extensively used in investigations on biological oxidations, and a brief description may be given here. (A fuller account of the technique and the results obtained by its use is given by Ahlgren (1926).) Methylene blue is an active reducible dye which can be used conveniently as the oxidiser in systems of this type. It becomes reduced to a colourless product:



(corresponding to the reduction of oxygen which we may write provisionally  $AH_2 + O = A + H_2O$ ), where  $AH_2$  is either spontaneously active or activated by a dehydrase. As  $MBH_2$  is rapidly reoxidised to methylene blue by oxygen, the reaction is carried out in special test tubes which can be evacuated (Thunberg tubes). For quantitative observations a given amount of methylene blue is added to the system under investigation, and the time taken for the disappearance of the blue colour, *i.e.*, for the reduction of that amount of methylene blue, is determined. As the reduction nearly always takes place at a constant velocity, the activity of the system is inversely proportional to the reduction time.

Thunberg added methylene blue to suspensions of minced animal tissues in neutral buffer solutions and determined the effect on the reduction time of adding various organic substances. Fresh animal tissues, however, rapidly reduce methylene blue without the addition of any oxidisable substance, since they already contain many substances activated by the dehydrases. Thunberg found, however, that by a preliminary extraction of the tissue with water these substances could be removed, and the tissue then lost the power of reducing methylene blue. Most of the dehydrases were however left intact in the tissue, and the reducing power could be restored by adding substances capable of being activated by them.

By such means Thunberg demonstrated the presence in frog muscle of dehydrases capable of oxidising lactic acid (and  $\alpha$  and  $\beta$  oxybutyric acids), succinic acid (but not glutaric or its homologues), malic, tartaric,  $\alpha$ -oxyglutaric, and citric acids, glutaminic acid, and glycerophosphoric acid. Glucose was not so oxidised. Dehydrases have since been found to be very widely distributed, not only in all animal tissues, but also in plant tissues, yeast cells, bacteria, etc.

Thunberg's work on frog muscle was repeated by Lipschitz (1921) using *m*-dinitrobenzene as an oxidiser instead of methylene blue. This becomes reduced to *m*-nitrophenyl-hydroxylamine. He found exactly the same results as Thunberg.

Several dehydrases have now been extracted from tissues and studied separately. Of these four have received most study, namely those which are responsible for the oxidation of succinic acid, purine bases and aldehydes, lactic acid, and citric acid.

*Specificity.* It is well known that enzymes frequently show a high degree of specificity towards their substrates. That is to say, a slight change in the structure of the molecule upon which they normally act (*e.g.* insertion of a methyl group or the like) is sufficient to prevent their action. The study of the specificity of the dehydrases is important not only from the point of view of enzyme action, but as giving information as to how many distinct dehydrases must be assumed to exist—whether a separate dehydrase exists for each substance oxidised or whether one dehydrase can oxidise many different substances.

The specificity of such systems—unlike the hydrolysing enzymes—must be studied from two different angles. Since two reactants (the “hydrogen donator” and the “hydrogen acceptor,” to use Thunberg’s terms) are concerned in each reaction, the specificity of the enzyme towards each must be separately considered. Therefore two series of experiments must be done; first taking a hydrogen acceptor known to be effective, and varying the nature of the hydrogen donator, to see how far the enzyme is specific towards the latter, and secondly taking a hydrogen donator known to be activated, and varying the hydrogen acceptor. Before such work can be done it is of course necessary for the enzymes to be separated from one another. This has been done only in a few cases and further work is much needed.

The few dehydrases which have been separated have been fairly thoroughly studied, and the main results are given below when dealing with the individual enzymes. Meanwhile it may be said that while probably no one believes in a separate dehydrase for every substance oxidised, there seems no doubt that there are quite a number of distinct dehydrases. Thunberg, working on chopped tissue, was not very successful in differentiating them, but later work has been more successful. Bernheim (1928), who discusses this question, shows that it is possible to obtain four different extracts (which may be called *A*, *B*, *C*, *D*), which behave as follows with methylene blue:

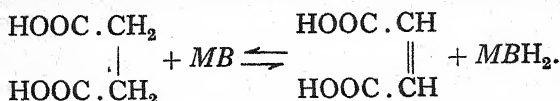
Extract	Succinic	Lactic	Citric	Aldehyde	Formic	Malic	Glutaminic, etc.
<i>A</i>	+	—	—	—	—	—	—
<i>B</i>	—	+	—	—	—	—	—
<i>C</i>	—	—	+	—	—	—	—
<i>D</i>	—	—	—	+	—	—	—

where + denotes rapid reduction on adding the substance at the head of the column and — denotes no reduction. This does not, of course, indicate absolute specificity, for instance, extract *B* probably oxidises  $\alpha$  hydroxy acids.

The important general fact has been brought out by studies on specificity that the dehydrases are very specific towards the substances they oxidise (the reducer) but practically not at all towards the oxidiser. This is precisely what we should expect if the reaction is one of type 2, where the enzyme acts on the reducer and not on the oxidiser, and this is indeed one of the main reasons for supposing that the dehydrases do act in that way. Examples of this are given below.

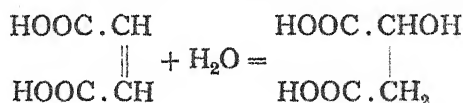
A few of the main points may now be mentioned about each of the best known dehydrases in turn.

*Succinoxidase.* This enzyme appears to be very active in all animal tissues. A solution may be made by extracting muscle thoroughly with water, and then extracting the residue with alkaline phosphate solution. Such an extract does not reduce methylene blue, etc., or absorb oxygen by itself; but, if sodium succinate is added, a rapid absorption of oxygen, or reduction of methylene blue, takes place, and the succinate becomes oxidised to fumarate (as shown by Einbeck, 1919), thus



This reaction with methylene blue is reversible, as shown by Quastel and Whetham (1924), and this is the only known case of a reversible dehydrase system: in presence of excess of fumarate reduced methylene blue becomes oxidised. Now fumarate cannot oxidise  $MBH_2$  in the absence of the enzyme, so it is clear that the latter activates both succinate and fumarate. Wieland's theory of activation in its original "hydrogen activation" form cannot account for the activation of fumarate, and must be extended on the lines indicated in the introduction.

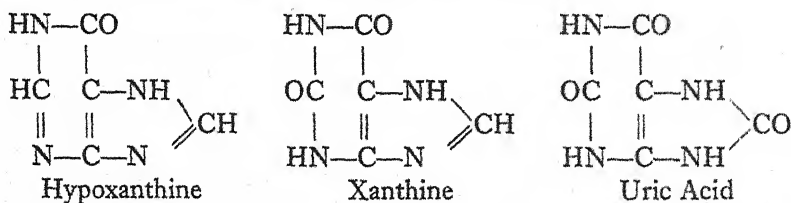
The fumarate formed is converted in the tissues into malate:



This is due to an enzyme known as "fumarase," which has recently been shown by Allwall (1928) in Thunberg's laboratory to be a distinct enzyme and not identical with the succinoxidase.

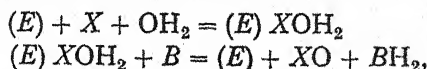
The specificity of the succinoxidase has not yet been thoroughly worked out. No substance has yet been found capable of replacing succinate as a hydrogen donor, although many related substances have been tested. It seems in fact to be specific for succinate. On the other hand, many other dyes, nitrobenzene, etc., can be substituted for methylene blue as oxidiser. Some special points relating to the behaviour of the system towards oxygen will be considered below.

*Xanthine oxidase.* This, which is the dehydrase about which most is known, oxidises the purine bases hypoxanthine and xanthine and is therefore the enzyme responsible for the production of uric acid in the body.



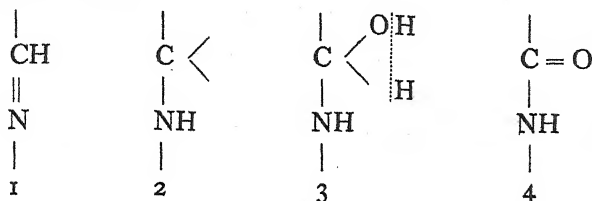
It has been known for some time that such an enzyme occurred in many animal tissues, but Morgan, Stewart and Hopkins (1922) found that it was present in considerable amounts in milk, from which source it is possible to separate and concentrate it greatly (Dixon and Thurlow, (1924, 1), Dixon and Kodama, (1926)). On the addition of hypoxanthine or xanthine to the enzyme, the system rapidly absorbs oxygen from the air in amount corresponding to the oxidation of the purine to uric acid, and uric acid can afterwards be isolated from the solution. The system also rapidly reduces methylene blue, in amount corresponding to the purine added.

The reaction is one of those cases in which the net result is a gain in oxygen atoms, so that on the Wieland view we must assume a hydrate of the purine, and write the reaction



where (*E*) represents the enzyme, *X* xanthine, and *XO* is uric acid. The xanthine becomes adsorbed and activated by the dehydrase, and thereupon forms a hydrate the active hydrogen atoms of which are taken up by the acceptor.

There is no need to assume that unactivated purine molecules must form hydrates. It may be that the activation itself enables the molecule to do so. For instance, the activation of the purine may be represented in harmony with Quastel's theory of activation by the change from state 1 to state 2 below (writing the essential part of the molecule only).



where the free valencies formed by the activation may well take up a water molecule to form an unstable hydrate 3, which loses hydrogen atoms to give the oxidation product 4. This would certainly give us a very clear picture of the reaction.

One or two points in the dynamics of the system may be mentioned (Dixon and Thurlow, 1924, 2). When the purine concentration is small, hypoxanthine reduces methylene blue exactly twice as fast as xanthine, and therefore, since the former must react with two methylene blue molecules in order to become uric acid and the latter with only one, uric acid is produced from both at the same rate. This means that, once the substrate comes into proper relation with the enzyme, two stages of oxidation may occur as easily as one.

The second point is that when the purine concentration is increased the reaction becomes much *slower*. This is a fairly common occurrence in surface reactions where one reactant is strongly adsorbed, and is due in this case to the formation of a film of purine over the enzyme surface which prevents the access of the methylene blue to the activated molecules. It seems to occur also when oxygen is used. This effect has been used in investigations on the specificity of the system, since it makes it possible to determine whether a given purine is adsorbed or not, even when it is not activated, by testing whether it inhibits the oxidation of a small amount of hypoxanthine. In high concentration xanthine and hypoxanthine reduce methylene blue at the *same* rate, as might be expected.

The specificity of the system has been studied by Dixon (1926) and in the purine series by Coombs (1927). Towards the hydrogen donor it is remarkably specific. With the exception of aldehydes (see below) nothing outside the purine series has been found to be oxidised, even slowly, although many substances have been tried, including all those known to be activated in the tissues. In the purine series only two others, very closely related to xanthine, are oxidised, namely 6, 8 dioxypurine and 2 thioxanthine. Insertion of a methyl or amino group in any position completely prevents activation, although it can be shown in many cases that the substance is still adsorbed. Coombs found that the adsorption is also remarkably specific, though

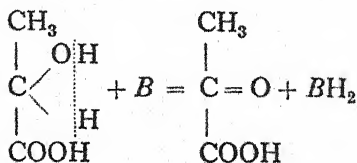


less so than the activation. He showed that only molecules closely conforming to the xanthine structure are adsorbed. For further interesting details his paper should be consulted.

The high degree of specificity in the purine series makes it all the more remarkable that this dehydrase is also an aldehyde oxidase. It will oxidise aldehydes to the corresponding acids just as readily as it will oxidise purine bases. (The evidence that the two oxidations are actually due to one enzyme is given by Dixon and Thurlow, 1924, 2.) It has of course been known for a long time that milk contains an aldehyde oxidase (known as Schardinger's enzyme, after its discoverer), and it is now believed that this is identical with the xanthine oxidase. Thus, in spite of the marked specificity above mentioned, the enzyme appears to have two quite distinct groups of substrates, and this fact undoubtedly somewhat complicates the situation. Nevertheless it is instructive to compare this specificity with that shown towards the hydrogen acceptor. Here no specificity can be observed at all—every active oxidiser seems to be capable of acting, provided it does not destroy the oxidase. For instance the following substances have been shown (Dixon, 1926) to oxidise hypoxanthine in presence of the enzyme (though not in its absence): methylene blue, indigos, indophenols, oxygen, hydrogen peroxide, alloxan, quinone, nitrates, chlorates, iodine, nitrobenzene, permanganate, etc. The same results are obtained with aldehyde instead of hypoxanthine. The contrast in behaviour is clear, and is exactly what we should expect if the oxidase activates the hydrogen donor only.

The distribution of the xanthine oxidase among animal tissues has been studied by Morgan (1926), and forms an interesting example of the curiously haphazard way in which many enzymes seem to occur. It is completely absent from the skeletal or cardiac muscle of all species. The liver contains large quantities in nearly all animals, but it is completely absent from the livers of the dog, hedgehog and pigeon. In the hedgehog it is present only in the small intestine and mammary gland. In ox, rat and birds the kidneys contain large amounts, but in all other species tested they contain none. And so on.

*Lactic oxidase.* Though, of course, this dehydrase occurs in animal tissues, it is most conveniently prepared from yeast (Bernheim, 1928). In presence of methylene blue or other dyes, nitrobenzene, etc., it oxidises lactates to pyruvates.



It apparently attacks  $\alpha$  hydroxy acids generally, but it appears to be quite specific for these and will activate none of the many other substances tested by Bernheim. An important fact is that the system is quite unable to use oxygen as an oxidiser, though it reacts freely with methylene blue. This point will be discussed later.

A similar enzyme has been obtained from bacteria by Stephenson (1928).

*Citric oxidase.* This was isolated, also by Bernheim (1928), from liver. In presence of methylene blue, etc., it readily oxidises citrates, but the nature of the product is not yet known. No other substance has been found capable of being oxidised by this dehydrase. Like the lactic oxidase, it is quite unable to react with molecular oxygen.

It exhibits, as shown by Bernheim, the same phenomenon as the xanthine oxidase, namely, increase of citrate concentration causes the reaction to proceed much more slowly. The citrate, like the purine, is strongly adsorbed by its enzyme, and forms a film which hinders the access of the methylene blue.

Further work on the dehydrases is much needed.

#### *Molecular oxygen as a hydrogen acceptor.*

We now come to the important question whether activation of the substance to be oxidised is alone sufficient to secure its oxidation by molecular oxygen, or whether an activation of the oxygen is also necessary. In other words, are the dehydrases concerned in the living cell with reactions of type 2 or type 4? Or again, is molecular oxygen capable of acting as a direct hydrogen acceptor?

The evidence suggests that while oxygen can undoubtedly act in this way with some systems, it cannot with others. Excluding the dehydrase systems, it is a general rule that systems which reduce methylene blue can also reduce oxygen without the addition of any extra catalyst—an important fact which must not be lost sight of. This would seem to indicate that “active hydrogen” can be taken up directly by molecular oxygen. For instance, the reduced forms of dyes (such as  $MBH_2$ ) are directly oxidised by molecular oxygen (see, *e.g.*, Harrison, 1927), even in the presence of cyanide, which is known to inhibit oxygen-activating systems (see later). There can be no question that the oxygen molecule is a direct acceptor of active hydrogen in this form, and other systems will be mentioned in the next section.

When we turn to the dehydrase systems we find that in some cases the same thing applies. In the oxidation of xanthine and of aldehydes, activation by the dehydrase is sufficient to render them readily oxidisable by molecular oxygen, and there is much evidence to show that no special oxygen-activating catalyst or carrier is concerned in the system. The addition of cyanide has no effect (Dixon and Thurlow, 1925), and the oxygen appears to be simply equivalent to the methylene blue as a hydrogen acceptor.

In other cases, however, the lactic and citric dehydrases, for instance, this is not the case. Here the activated molecules, which are freely oxidised by methylene blue, are quite unable to react with molecular oxygen. The reason for this important difference is unknown. The addition of a small amount of methylene blue will enable the system to react with oxygen, because the methylene blue acts as an intermediate “carrier” between the activated reducer molecules and the oxygen.

The case of the succinoxidase is particularly interesting. As mentioned above, a solution of succinoxidase plus succinate rapidly absorbs oxygen and reduces methylene blue. The addition of a very small amount of cyanide however completely

abolishes the oxygen uptake, while it leaves the reduction of methylene blue unaffected (Thunberg, 1917; Fleisch, 1924). The cyanide clearly cannot be preventing the activation of the succinate, for then it would affect the methylene blue reduction. Therefore oxygen must be unable to react with activated succinate molecules, and the cyanide acts by inactivating either some carrier, or a catalyst particularly concerned with oxygen, present in the solution. The nature of this other system has been made clear by very recent work by Keilin, which will be described in a later section. Dixon and Thurlow (1925) found that the oxidation of hypoxanthine in liver was completely unaffected by the addition of cyanide, which, in the same sample of tissue, completely prevented the oxygen uptake due to succinate.

On the basis of the failure of the lactic oxidase system, etc., to react with oxygen, Szent-Györgyi (1925, 1) put forward the theory that active hydrogen reacts only with *active* oxygen. This view must be regarded as not wholly satisfactory, since it ignores those systems which do not require oxygen activation.

It is quite clear that we must divide the dehydrase systems into two classes. One, represented by the xanthine oxidase, can react directly with molecular oxygen; the other, represented by the lactic oxidase, etc., can utilise oxygen only by the co-operation of some extra system. For want of a better term we may call the first class "aerobic dehydrases," and the second "anaerobic dehydrases," although it must be remembered that the aerobic dehydrases can also act anaerobically.

### III. PEROXIDE FORMATION, PEROXIDASES AND COUPLED OXIDATIONS.

The powerful catalytic action of traces of ferrous salts on the oxidation of many organic substances by hydrogen peroxide is well known. It has been pointed out by Dakin (1922) that many of the oxidations known to occur during metabolism are also brought about by hydrogen peroxide plus a trace of ferrous salt. The parallelism is striking in many cases. It has been known since the time of Schönbein that very active catalysts, whose action resembles that of the ferrous salt, are widely distributed in plant and animal tissues. Enzyme catalysts of this nature are known as "peroxidases," and as they activate the oxidiser (the peroxide) in the reactions they bring about, such reactions are of type 3. They have been principally studied by the substitution method (2 a) in which some reagent which gives a colour on oxidation is substituted for the substance normally oxidised. By far the greater part of the investigation has been done on peroxidases of vegetable origin, and the animal peroxidases have been comparatively very little studied.

Interest in peroxidase systems was aroused very largely by the work and views of Bach and Chodat (1903). As this work was carried out on vegetable systems, only a very brief outline will be given here.

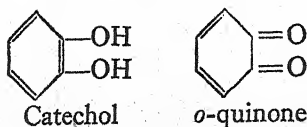
Many plants had been shown to give the guaiacum reaction. That is to say, on addition of tincture of guaiacum an intense blue colour is produced in the presence of oxygen. The guaiacum contains guaiaconic acid, which on oxidation produces the blue pigment; so that it is a test for the activation of the oxidiser, and is therefore the converse of methylene blue which is a test for the activation of the reducer.

As a result of their work, Bach and Chodat came to the conclusion that the bluing of guaiacum was due to the co-operation of two distinct enzymes, namely a peroxidase, and a peroxide-forming enzyme which they termed "oxygenase." The latter was assumed to combine with molecular oxygen forming an organic peroxide which was then activated by the peroxidase to bring about the oxidation. The oxygenase was shown to produce oxidising substances in presence of oxygen, but as a matter of fact there was very little evidence as to the nature of these substances. It had been shown that neither the peroxidase alone nor hydrogen peroxide alone could oxidise guaiacum, but peroxide in the presence of peroxidase produced a very rapid oxidation.

The Bach-Chodat theory brought into prominence the idea of successive reactions which has been of great value in dealing with biological oxidations: according to this view the actual oxidation which was brought about by the peroxidase was dependent on another reaction—the formation of peroxide by "oxygenase."

M. W. Onslow (1919, 1920) later showed that the oxygenase of plants could be resolved into two factors: an enzyme (which she termed simply "oxygenase") and an oxidisable organic substance, which proved to be catechol (or a derivative). After the removal of the catechol substance the enzyme was no longer able to produce peroxide-like substances in presence of oxygen, but on the addition of a small amount of catechol this became rapidly oxidised by oxygen with the production of such substances. The action of this enzyme is thus simply to bring about the oxidation of catechol by oxygen. (In the absence of any definite knowledge as to the mechanism of its action, and since it seems probable from other evidence that it does not act merely on oxygen, it seems that the term "oxygenase," besides being ambiguous, is misleading; and the name "catechol oxidase," which assumes nothing as to its mode of action, is preferable and will be used here.<sup>1</sup>)

The nature of the oxidising substance produced by the action of the catechol oxidase was not clear until the work of Szent-Györgyi (1925, 2), which also showed that the system did not act wholly in accordance with Bach and Chodat's theory. Szent-Györgyi found in fact that the substance formed was not a true peroxide, and also that the presence of peroxidase was unnecessary for the oxidation of the guaiacum. He added catechol to a purified catechol oxidase preparation and allowed the oxidation of the catechol to proceed. Then he removed the enzyme by precipitation and tested the enzyme-free solution with guaiacum, which gave immediately a deep blue colour, showing that peroxidase was unnecessary for the action of the oxidising substance. The most probable oxidation product of catechol is ortho-quinone,



<sup>1</sup> Raper (1928) proposes that it should be called simply "tyrosinase"; but since the oxidation of tyrosine seems to require the presence of other factors, this does not appear desirable. Purified catechol oxidase has very little action on tyrosine.

and Szent-Györgyi found that the properties of the substance formed by the catechol oxidase corresponded closely with those of *o*-quinone. Further, he found that *o*-quinone readily blued guaiacum in the absence of peroxidase. By oxidising the guaiacum the quinone is of course reduced back to catechol.

The work of Pugh and Raper (1927) actually established that the substance formed was *o*-quinone itself.

The possibility must be considered that hydrogen peroxide, as well as the quinone, is produced in this reaction. For Wieland and Fischer (1926) isolated from the fungus *Lactarius* a catalyst which oxidised catechol by oxygen with the production of both substances according to the equation



Solutions of catechol, even with no added catalyst, oxidise slowly in the air, apparently according to the above equation (Onslow and Robinson, 1926). As however *o*-quinone is very unstable, the resulting solution will oxidise guaiacum only after the addition of peroxidase.

The catalyst of Wieland and Fischer appeared to be of a different nature from the catechol oxidase proper. It was not an enzyme, it was thermostable, and differed in many other essential respects from the oxidase. Later Wieland and Sutter (1928) obtained from *Lactarius* a true thermolabile oxidase, and this oxidised catechol readily but produced no peroxide in doing so.

On the other hand, Onslow and Robinson (1926) have obtained some evidence of the production of small amounts of hydrogen peroxide during the oxidation of catechol by the plant oxidase. Kortschewsky (1929) has obtained an "oxygenase" from *Aspergillus*, which apparently produces hydrogen peroxide.

Thus the evidence seems to show that while the possibility of the formation of small amounts of peroxide by this system and subsequent oxidation by peroxidase cannot be neglected, much the greater part of the oxidation is due to the quinone without the action of peroxidase. Pugh (1929) has recently given a critical discussion of Bach and Chodat's work, and found no evidence of the dual nature of the system postulated by them.

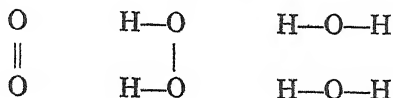
Although the catechol oxidase, which apparently does not occur in the tissues of the higher animals, thus fails to illustrate their theory, systems which form hydrogen peroxide are known to exist in animal tissues, as will be shown below. The existence of such systems indeed is made probable by the very existence of the peroxidases, since these would be useless in the absence of a supply of peroxide. Thus it appears that some oxidations are brought about in the tissues by the "oxygenase"-peroxidase type of coupled system.

Systems of this type would appear to have little connection with the dehydrase type of system, and at first sight they would seem to represent two entirely distinct and independent oxidation mechanisms occurring in the tissues. The interesting and important work of Thurlow has however revealed the connection between the two mechanisms.

Before discussing this it will be necessary to make a short digression and consider a further aspect of the behaviour of molecular oxygen as a hydrogen acceptor.

*The reduction of the oxygen molecule.*

When oxygen acts as a direct hydrogen acceptor, becoming reduced ultimately to water, one would expect this reduction to take place in two stages. In the first stage two hydrogen atoms would be taken up with the "breaking of a bond" between the two oxygen atoms. In the second stage a further pair of hydrogen atoms would be taken up with the disappearance of the remaining bond thus:



The first reaction therefore results in the production of hydrogen peroxide, which in its turn acts as a hydrogen acceptor and becomes reduced to water.

Let us consider the reaction  $2\text{H} + \text{O}_2 \rightarrow \text{H}_2\text{O}_2$  (where  $2\text{H}$  represents two active hydrogen atoms (in Wieland's sense), occurring either in an organic molecule or loosely attached to a metallic surface, etc.), and let us see how the various kinds of active hydrogen behave towards molecular oxygen.

Atomic hydrogen itself, which may be regarded as active hydrogen *par excellence*, has been shown (Taylor, 1926) to react with oxygen to give hydrogen peroxide.

The same is true of the hydrogen taken up by platinum. The so-called "nascent hydrogen" is merely another form of active hydrogen (in this connection see Dixon, 1927, 1). Metals dissolving in acids, or metals with a high electrolytic solution pressure suspended in water (*e.g.*, zinc dust), become charged with active hydrogen, and if the solution contains dissolved oxygen, hydrogen peroxide is formed.

A well-known electrolysis experiment also illustrates the reaction. If a dilute electrolyte solution is electrolysed by passing a current between electrodes, and if the solution contains dissolved oxygen, some of the hydrogen liberated by the current at the cathode reacts with the oxygen to form hydrogen peroxide. If the amount of oxygen in the liquid is much increased by exposing it to oxygen under high pressure, the hydrogen is no longer evolved as hydrogen gas, but is converted into hydrogen peroxide.

In those cases where the reaction takes place at a metal surface the possibility that the surface may have an activating effect on the oxygen cannot however be altogether neglected.

The production of hydrogen peroxide has also been shown in a number of cases where the active hydrogen atoms are contained in organic molecules. Such molecules are, as has been mentioned above, in general "autoxidisable" (*i.e.*, spontaneously oxidisable by molecular oxygen); and the production of hydrogen peroxide during the autoxidation of organic substances seems to be of quite general occurrence. There are indications that it takes place in many cases besides those in which its formation has been definitely established.

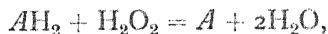
The autoxidation of polyphenols such as catechol has been mentioned. Other cases in which hydrogen peroxide has been shown to be formed are the autoxidation of the reduced forms of dyes such as indigo and probably methylene blue, hydrazo-



benzene, hydrazine, thiophenol and a few other substances. It appears that we may write such reactions



in accordance with the Wieland theory. This is, of course, followed by the reaction



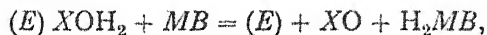
and the amount of peroxide present will depend on the relative velocities of these two reactions. In some cases, *e.g.*, the autoxidation of cysteine, discussed later, the amounts of peroxide formed are too small to be detected by chemical means, as the second reaction is rapid. There is, nevertheless, some evidence of its formation.

It will be seen therefore that there are good grounds for believing that *when molecular oxygen acts as a direct hydrogen acceptor, hydrogen peroxide is formed.*

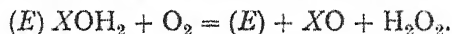
The process can be pictured equally well on the electron transfer theory. If the oxygen molecule takes up an "electron pair,"  $O_2$  will result, and this is the double anion of hydrogen peroxide.

#### *Dehydrase—peroxidase systems.*

We are thus brought to the important work of Thurlow (1925). It has been mentioned that one class of dehydrases can utilise oxygen as a direct hydrogen acceptor, and from what has just been said it would be expected that hydrogen peroxide would be formed in the process. Thurlow, working on the xanthine and aldehyde oxidase systems, actually found that considerable quantities were produced, and could be detected by a variety of chemical tests, including the titanium test. In dealing with xanthine oxidase in a previous section the reaction with methylene blue was written



and the reaction with oxygen may be similarly written



Thurlow found that when hypoxanthine or xanthine was added to a solution of xanthine oxidase, and shaken with air, peroxide could very soon be detected in the solution, and as the oxidation of the purine proceeded the amount of peroxide present rapidly increased. After some time, however, a maximum was reached, and the peroxide then gradually disappeared from the solution. This disappearance has been shown to be due to a reaction



Exactly the same results were obtained using aldehyde in place of hypoxanthine.

Now, according to Bach's definition of an "oxygenase" as a system capable of converting molecular oxygen into a peroxide, it will be seen that such a dehydrase plus its substrate is an oxygenase. Therefore, if a peroxidase is added to this system, the complete system which will oxidise guaiacum in the presence of oxygen should be produced. Thurlow showed that this was the case. On adding guaiacum to an aerated solution containing xanthine oxidase plus hypoxanthine plus (animal or vegetable) peroxidase a deep blue colour was at once produced, whereas if any one of the constituents were omitted from the system no colour was obtained.

Thurlow found that for studying the system it was preferable to use nitrite in the place of guaiacum. In the presence of animal peroxidases hydrogen peroxide readily oxidises nitrite to nitrate, and the amount of nitrite remaining unoxidised can be easily estimated colorimetrically by the use of the Griess-Ilosvay reagent. Vegetable peroxidases are not suitable for use with nitrite, and Thurlow used peroxidases prepared from milk or liver. The following experiment was typical of the results she obtained. To four tubes were added solutions of the substances indicated in the second column of the following table, together with an amount of nitrite in each sufficient to give a colour test of a strength represented by ++++. Air was then bubbled through the solutions for a short time, after which they were found to give the results shown.

Tube	Contents	Nitrite remaining unoxidised
1	Xanthine oxidase + xanthine	+
2	Xanthine + peroxidase	+
3	Xanthine oxidase + peroxidase	+
4	Xanthine oxidase + xanthine + peroxidase	—

Thus no oxidation of nitrite took place except in tube 4 where all the constituents were present, and here it was rapidly and completely oxidised.

Szent-Györgyi (1928) has recently confirmed these results, using adrenaline, which becomes oxidised to a coloured product, instead of nitrite. The presence of all the constituents is of course necessary for this oxidation also.

The work was extended somewhat by Harrison and Thurlow (1926) who showed that the complete system also brought about the oxidation of some ether-soluble substance present in milk. It seems very desirable that further work should be done on the system in order to determine what substances of biological interest are oxidised by it.

The work of Thurlow brings into relation with one another two types of oxidising enzyme which previously appeared to be quite unconnected. Of course it applies only to the class of dehydrases which are capable of reacting with molecular oxygen (the aerobic dehydrases). The others react with quite a different type of system which will be considered later.

Systems in which the oxidation of one substance depends upon the oxidation of another are known as coupled systems, and the process as coupled oxidation. In this case the peroxide which oxidises the nitrite, etc., is itself formed by the oxidation of the xanthine. The nitrite oxidation therefore depends on the oxidation of the xanthine in such a way that at most only one molecule of nitrite can be oxidised for each molecule of xanthine oxidised. This is a characteristic property of coupled systems.

Other systems capable of forming peroxide, and giving rise to coupled oxidations in the presence of peroxidase, are known to occur in animal tissues, the tyramine oxidase mentioned below, for instance, which also appears to react directly with oxygen.

*Peroxidases*

Some facts relating to the peroxidases themselves must now be considered. They are most easily obtained from vegetable sources, and by far the greater part of the work has been carried out on vegetable peroxidases. The work of Willstätter on the purification of vegetable peroxidases is, of course, classical. Unfortunately, comparatively little study has been made of the animal peroxidases.

Whether they are iron compounds is not yet known. Their action closely resembles that of the organic iron compounds related to haematin which occur in the tissues, but there appear to be certain slight differences in specificity. The presence of the thermostable haematin compounds, which have been called "pseudo-peroxidases" tends to mask the action of the peroxidases proper, but there seems little doubt that true thermolabile peroxidases do occur in animal tissues, and such a peroxidase has been prepared from milk by Thurlow (1925). It is possible, however, that the greater part of the oxidations of the peroxidase type in the tissues may be actually due to the haematin compounds. Harrison and Thurlow (1926) showed that ferrous salts, haematin, etc., were capable of acting as coupled systems with the xanthine oxidase; they found indeed that with iron instead of milk peroxidase the system was able to oxidise lactic and  $\beta$ -hydroxybutyric acids, which it was unable to do with milk peroxidase. (The lactic acid was not oxidised to pyruvic acid, as in the lactic dehydrase system, but to acetaldehyde; and the hydroxybutyric to acetoacetic acid.) On the other hand, there are slight indications that the true peroxidase may act on substances which are not acted on by haematin.

The peroxidases have been studied almost entirely by following the oxidation of one or other of the "peroxidase reagents" by hydrogen peroxide in their presence. This is very regrettable, since the peroxidase reagents are artificial substitutes for the substances normally oxidised, and the result is that we have practically no knowledge as to the nature of the substances actually oxidised by them *in vivo*. Experiments to determine this (now in progress) may be expected to lead to interesting results.

With regard to the mechanism of peroxidase action, the theory of Kastle and Loevenhart (1903) still seems to be fairly generally accepted. According to this theory the peroxidase combines with the peroxide to give a compound resembling an organic peroxide which is active and brings about the oxidations. This is supported by the fact that most organic peroxides seem to be active. Benzoyl peroxide, for example, is almost exactly equivalent in its behaviour to peroxidase plus hydrogen peroxide: it will oxidise the peroxidase reagents benzidine, guaiacum, nitrite, etc., rapidly without the addition of any catalyst, and the addition of peroxidase does not seem to accelerate the reaction.

*Specificity.* It is interesting to compare the specificity of a system like peroxidase, in which the oxidiser is activated, with that of the dehydrases, where the reducer is activated. There it was pointed out that the catalyst was specific towards the reducer and the exact nature of the oxidiser was apparently immaterial. Here the

reverse is the case, and it is the nature of the reducer which is comparatively unimportant.

The specificity of peroxidases towards the oxidiser has not been well worked out. The action appears to be limited to substances containing a peroxide group, but whether organic peroxides, as well as hydrogen peroxide, can act is not definitely settled. Investigation is made difficult by two facts: most of the organic peroxides are already active and oxidise the reagent in the absence of peroxidase, and they hydrolyse fairly rapidly in solution with the production of hydrogen peroxide. The writer has recently obtained indications that persulphate is activated by animal (though not vegetable) peroxidases, and it is hoped to investigate this more thoroughly.

With regard to the reducer it is only necessary to say that the oxidation of the following substances has been used as a means of investigating peroxidase action: guaiacum, guaiacol, polyphenols, hydroquinone, catechol and pyrogallol, nitrite, the phenylenediamines, the cresols, benzidine, leuco-malachite green, phenolphthalin, hydriodic acid, the "nadi" reagent ( $\alpha$  naphthol + dimethylparaphenylenediamine) and many others. There is as little specificity observable here as in the case of the hydrogen acceptor with the xanthine oxidase.

These facts show that in peroxidase systems it is the oxidiser which is activated. Wieland's hydrogen-activation theory does not therefore apply to peroxidase action, although naturally the reaction may take place in accordance with his hydrogen transfer theory.

*Hexuronic acid.* This substance, which is an active isomer of glycuronic acid, has recently been isolated from plant tissues and also from the cortex of the adrenal gland by Szent-Györgyi (1928), who believes that it may be specially related to peroxidase systems. It exists in an oxidised and a reduced form. The latter is very rapidly oxidised by quinones, and as polyphenols such as catechol are oxidised to their corresponding quinones by peroxide plus peroxidase, this system in presence of a trace of catechol very rapidly oxidises hexuronic acid. The hexuronic acid does not appear to be oxidised at an appreciable rate by peroxide plus peroxidase alone. The oxidised form does not seem to be reduced by the dehydrase systems, but is reduced by the glutathione system, and Szent-Györgyi suggests that it forms a carrier between that system and the peroxidases. The significance of such a link seems however a little obscure. It will be referred to again in discussing the glutathione system, but it may be pointed out here that hexuronic acid only occurs in traces, if at all, in animal tissues other than the adrenal cortex. In plant tissues it can hardly fail to have considerable importance in peroxidase systems in view of the facts given in Szent-Györgyi's paper.

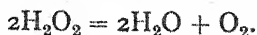
*Other oxidases.* The catechol oxidase is a member of a class of enzymes, mostly of vegetable origin, which includes the polyphenolases, laccases, tyrosinase, etc. These enzymes are as yet very ill-defined as a rule, largely because in many cases the individual enzymes have not been sufficiently separated, so that several of the systems are probably not single enzymes but mixtures of enzymes. Other facts which have tended to complicate the position are that in several cases, catechol for instance, the oxidation is also brought about by peroxidase, which may also be

present in the preparation; and that most investigators have not measured the uptake of oxygen, but have observed the production of the dark coloured products (melanins) which are formed as the ultimate product of the reactions. The production of these substances is not always a reliable guide to the amount of oxidation taking place. These enzymes have been reviewed by Raper (1928) and they will not be dealt with in detail here. With one or two exceptions they do not seem to occur in animal tissues. The tyrosinase system occurs in certain invertebrates, and seems to be connected with pigment formation from tyrosine. According to Raper, a second phenolic group is first introduced into the tyrosine in the ortho position to the first. The resulting dioxyphenylalanine ("dopa") is then oxidised, in a manner analogous to catechol, to the corresponding quinone, which is unstable and becomes rapidly converted into the dark coloured product. Tyrosinase seems to be absent from the higher animals, but the skin of certain animals contains "dopa" oxidase which brings about the second stage of the above process. The indophenol oxidase, which oxidises among other things the "nadi" reagent and *p*-phenylenediamine, and is so widely distributed in animal tissues, logically belongs here; but on account of its great importance in connection with the cytochrome system it is dealt with in a special section later.

All these enzymes have one characteristic in common. Unlike the dehydrases, but like the peroxidases, they are very sensitive to cyanide. The presence of a very small amount of cyanide is sufficient to stop their action completely.

We do not yet know the significance of the interesting tyramine oxidase recently discovered in liver by Hare (1928). This enzyme rapidly oxidises tyramine and phenylethylamine, with the uptake of one molecule of oxygen for each molecule oxidised, but does not act upon tyrosine, "dopa," *p*-cresol, adrenaline, etc. It differs completely from the enzymes mentioned above, in that cyanide has absolutely no effect on the oxygen uptake. Hare also showed that hydrogen peroxide is formed in the reaction. The system is in fact, like the xanthine oxidase, an "oxygenase," and on the addition of milk peroxidase she found that coupled oxidations could be brought about. The oxygen seems to act as a direct hydrogen acceptor in this system also. Methylene blue did not act as a hydrogen acceptor, but it is possible that the energy conditions did not permit of this.

*Catalase.* Hydrogen peroxide except in very small concentration is extremely toxic to most enzymes. If, therefore, peroxide-producing systems exist in the cell it would seem that some protective mechanism would be necessary to keep the concentration of peroxide from becoming too large. This is the function of the enzyme catalase, which is almost universally distributed in the tissues. This enzyme decomposes hydrogen peroxide with the production of *molecular* oxygen thus:



It was discovered by Löw in 1901.

Its protective action can be well seen with the xanthine-oxidase system, as shown by Dixon (1925). During the aerobic oxidation of hypoxanthine by a preparation of xanthine oxidase the peroxide which is formed progressively destroys the oxidase

itself. If only a small amount of oxidase is taken it may be completely destroyed after only a small fraction of the hypoxanthine has been oxidised. The reaction then of course ceases, and the solution when tested with methylene blue is found to be quite inactive. If, however, some catalase is added at the beginning of the reaction it decomposes the peroxide formed and the destruction no longer occurs. The oxygen uptake proceeds with a practically constant velocity until the hypoxanthine is all oxidised, and on subsequently testing the solution with methylene blue (and fresh hypoxanthine) it is found to be still active.

It might be thought that if the peroxide formed is destroyed by catalase the coupled oxidations with peroxidase would be prevented. This is not the case, however, as shown by Thurlow (1925). The effect of the catalase is to keep the peroxide concentration down to small values, but peroxidase is capable of acting with very small concentrations of peroxide. Thurlow found that, although it was true that the presence of catalase somewhat diminished the rate of the coupled oxidation, this continued to take place no matter how much catalase was added.

The action of catalase appears to be restricted to hydrogen peroxide, and it is generally stated to have no action on organic peroxides. It must be admitted, however, that only a small number of organic peroxides has been tested with the enzyme.

Catalase, like peroxidase, etc., is poisoned by small amounts of cyanide.

#### IV. WARBURG'S THEORY AND THE "RESPIRATORY ENZYME."

It is now time to consider a theory of cell respiration which has assumed considerable prominence during the past few years. Warburg's<sup>1</sup> theory is in brief that respiration (the utilisation of molecular oxygen by the living cell) is a process which is due to catalysis by iron. He has summarised the essential point of the theory in the following words (Warburg, 1923): "According to the theory the catalyst of respiration is iron... The primary reaction of respiration is the reaction between molecular oxygen and iron, and molecular oxygen can only react in the cell by this reaction, and not with the organic molecules."

This would definitely exclude the possibility of molecular oxygen acting as a direct hydrogen acceptor in accordance with the Wieland view. The two theories are definitely contradictory, as Warburg points out, in respect to the manner in which molecular oxygen acts. Wieland regards the activation of hydrogen, or rather of the molecules to be oxidised, as the important event. Warburg, on the other hand, regards the activation of oxygen by iron as the essential process, and considers the activation of the organic molecules as of secondary importance. (The two theories have been discussed in relation to one another by Hopkins (1926).)

According to the Warburg view, the uptake of oxygen by the cell is to be regarded not as the sum total of the separate uptakes of a number of distinct oxidase systems of different types, but as one process due to the action of one catalytic mechanism.

With regard to the nature of the catalytic iron, Warburg states that the free ions

<sup>1</sup> The papers of Warburg and his collaborators on this subject from 1912 to 1927 have been republished in the form of a book: *Über die katalytischen Wirkungen der lebendigen Substanz* by Otto Warburg, Berlin, 1928.



are inactive and that the active iron is present in the form of a definite complex compound. This catalytic compound, whose action is responsible for respiration as a whole, Warburg calls "the respiratory enzyme" (*das Atmungsferment*); and as a result of his later work he believes it to be a compound related to the haematin series of pigments.

Warburg has put forward two theories of the mechanism by which the iron acts. According to the earlier view the compound can exist in two states of oxidation. The organic molecules reduce the oxidised form, becoming thereby themselves oxidised; and the resulting reduced form of the compound is then reoxidised by molecular oxygen (Warburg, 1923). It will be seen that, according to this form of the theory, the iron acts simply as an intermediate carrier, of the type referred to in the introduction, between oxygen and the organic molecules oxidised. Although this process is frequently referred to as oxygen activation by iron, this is incorrect. The process is an iron catalysis, but there is no reason why the iron should be regarded as activating the oxygen rather than the organic molecules. It would be just as logical to say that the iron activated the organic molecules.

The more recent theory (Warburg, 1927) assumes that the "respiratory enzyme" combines with oxygen thus:



where  $X.Fe$  represents the "respiratory enzyme." The oxidation of the organic substances then follows:



Warburg leaves it undecided whether activation of the organic molecules is also necessary.

This view, which involves an actual oxygen activation, resembles the theory of Bach in many ways.

A brief summary will now be given of the evidence upon which Warburg's theory is based.

In the first place, Warburg (1921) studied the inhibitory effect of a large number of narcotic substances on the velocity of cell respiration. Among other substances alcohols, ketones, urethanes, amides, substituted ureas, etc., were studied. As the result of a considerable number of measurements he came to the conclusion that the inhibitory power of these substances was proportional to their adsorption on the active intracellular surfaces where respiration took place. In other words, these substances acted simply by blocking the active surfaces and preventing the access of the normal reactants. One or two substances however exerted an inhibiting action out of all proportion to their adsorption. Examples of these were HCN and  $H_2S$ . HCN in a concentration of  $M/10,000$  has a definite inhibitory effect on the respiration of most cells, and this effect is about 10,000 times what would be predicted from its adsorption. Now the substances which acted thus were known to inhibit strongly most reactions catalysed by iron.

Next Warburg (*loc. cit.*) studied the behaviour of a "cell model." He found that a suspension of blood charcoal (*i.e.*, charcoal prepared by heating blood) in

water was capable of oxidising, by means of molecular oxygen, many substances, especially oxalic acid and certain amino acids. That is to say, a suspension of blood charcoal in an oxalic acid solution "respired." Now the action of narcotics on this system was found to be closely similar to their action on the cell. Narcotics such as alcohol, etc., again produced an inhibition proportional to the amount of surface blocked, and again cyanide, etc., were infinitely more powerful.

Blood charcoal naturally contains a certain amount of iron, and Warburg believed that the catalytic power of the charcoal was due to this. He confirmed this (1924) by preparing iron-free charcoal by heating a mixture of pure cane sugar and silicate. This proved to be quite inactive. If iron salts were added to the mixture before heating inactive charcoals were still obtained, but if both iron and some organic nitrogen compound were added, the resulting charcoals were very active. Warburg concluded that the activity was due not to the charcoal surface itself, but to iron-nitrogen-carbon complexes in the surface, and that the HCN acted by selectively poisoning these active centres by combining with the iron atoms.

The question of charcoal activity however is not a simple one; for instance, by heating cane sugar without silicate Warburg obtained iron-free charcoals having quite a moderate activity, and HCN had no effect on these.

From the analogy between the behaviour of the "cell-model" and the cell Warburg concluded that respiration was a surface reaction depending on iron catalysis.

Warburg found that a small amount of cyanide completely abolished the respiration of sea urchin eggs, yeast cells, and bird's red blood corpuscles.

He also quotes as evidence of the necessity of oxygen activation by iron the fact mentioned previously that a small amount of cyanide completely prevents the oxidation of succinic acid by oxygen in presence of the succinoxidase, while leaving its oxidation by methylene blue unaffected. He regards methylene blue as being equivalent to oxygen plus iron.

Warburg (1926, 1927) found that carbon monoxide also inhibited cell respiration, and his evidence for the relationship of the "respiratory enzyme" with haematin arises chiefly from work on this effect. It is well known that carbon monoxide forms addition compounds with many iron compounds related to haematin (*e.g.*, haemoglobin), and that these compounds are dissociated by light. Warburg found that the marked inhibition of the respiration of yeast produced by CO was almost entirely abolished by light, which was further evidence in favour of the theory. Light had no effect on the respiration of yeast in the absence of CO.

Warburg (1928) made use of this effect of light to determine the spectrum of the CO compound of the "respiratory enzyme." By determining the effectiveness of light of different wave-lengths in restoring the respiration inhibited by CO, he was able to obtain a curve showing how the effectiveness varied with the wave-length. The natural assumption is that the light must be absorbed by the CO "respiratory enzyme" compound if it is to be effective in dissociating it, and in that case the curve must represent the actual absorption spectrum of the compound. The curve actually obtained closely resembled the spectrum of a CO-haemochromogen. This is the main reason for believing the "respiratory enzyme" to be a haematin derivative.

There appears to be a definite competition between carbon monoxide and oxygen for the "respiratory enzyme." That is to say, if a certain degree of inhibition is produced with a given oxygen pressure by a given pressure of CO, increasing the oxygen pressure lessens the inhibition, and it is necessary to increase the CO pressure in a corresponding ratio in order to obtain the same inhibition as before. The ratio  $\text{CO}/\text{O}_2$  controls the inhibition, not the CO pressure itself. This competition indicates that the CO combines with the "respiratory enzyme" in the same way as the oxygen, so that we may write



where the effect of light is to alter the equilibrium towards the left-hand side of the equation. On the basis of a large number of measurements Warburg has worked out a constant expressing the relative affinity of the "respiratory enzyme" for oxygen and CO. This is defined as  $k = \frac{n}{1-n} \cdot \text{CO}/\text{O}_2$ , where  $n$  is the ratio of the respiration in presence of CO to the respiration in its absence. A small value of  $k$  thus corresponds to a high affinity for CO, and *vice versa*. The value for yeast in the dark was found to be of the order of 10 (it varied in different experiments between 4 and 13), and in the light about 116.

The above is a brief account of the main evidence in support of Warburg's theory of cell respiration. In addition it may be mentioned that he admits that such oxidations, as, for instance, leuco-methylene blue, are true autoxidations, but denies that naturally occurring substances are autoxidisable. In support of this denial he cites two cases of apparent autoxidation which have proved to be actually due to iron catalysis. One of these is the oxidation of fructose in phosphate solutions, which was shown by Meyerhof and Matsuoka (1924) to be dependent on the occurrence of traces of iron in the system. The reaction is greatly accelerated by the addition of very small amounts of iron or copper salts, and is strongly inhibited by cyanide. The other case is the oxidation of substances containing the sulphydryl ( $-\text{SH}$ ) group, such as cysteine and glutathione. This had been thought to be a true autoxidation, and considerable importance had been ascribed to it in connection with biological oxidations, but it was shown by Warburg and Sakuma (1923) that this also was an iron catalysis. The system will be discussed in a later section, but it may be mentioned here that they found that the "autoxidation" of cysteine was prevented by cyanide, one molecule of cyanide being able to prevent the oxidation of many thousand molecules of cysteine, and that the addition of minute traces of iron or copper enormously accelerated the reaction. They also found that really pure preparations of cysteine were not autoxidisable, but became so on the addition of traces of metal salts. The amounts of iron or copper necessary are of course excessively small. These experiments were repeated on glutathione by Harrison (1924), who obtained similar results.

Such is the evidence for Warburg's theory. A few points of criticism may however be brought against the evidence, and these will now be briefly considered.

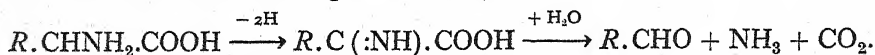
It is quite clear that the idea of a catalytic system, poisoned by cyanide, CO, etc., and responsible for, at any rate, a large part of the oxygen uptake of the cell, is justified.

(Wieland endeavoured to explain the action of cyanide by a poisoning of the catalase and consequent destruction of the oxidases by the accumulation of peroxide, but this view is totally inadequate and has been refuted.) Warburg's claim that this "respiratory enzyme" accounts for the whole respiration of animal tissues cannot however be allowed, in view of the recent work of Dixon and Elliott (1929). They studied the effect of varying concentrations of HCN on the respiration of a considerable number of typical animal tissues, and found that the maximum inhibition obtainable with HCN varied in different tissues between 40 and 90 per cent., with an average of about 60 per cent. The maximum inhibition was usually already produced by  $M/1000$  HCN, and increasing the concentration 100 times produced no greater inhibition. It is clear, therefore, that the "respiratory enzyme," which is highly sensitive to cyanide, can at most only account for about 60 per cent. of the respiration of animal tissues, and that one-third of the respiration is due to cyanide-stable systems. Dixon and Elliott confirm Warburg in finding that the respiration of yeast is completely inhibited by cyanide, so that in yeast the "respiratory enzyme" may account for practically the whole respiration.

Further, there is one organism—*Chlorella*—whose respiration is unaffected by HCN, as shown by Emerson (1927). It becomes somewhat cyanide-sensitive, however, if supplied with glucose.

With regard to the charcoal model, Wieland points out that, so far from being able to bring about the numerous oxidations occurring in the cell, its action is in reality very limited. It will oxidise only amino acids (and a few substances such as oxalic acid) and does not oxidise fatty acids, glucose, succinic acid, etc.

Wieland (1924) studied the oxidation of amino acids by charcoal in greater detail. Warburg supposed that the amino acids were completely burnt to  $\text{CO}_2$ , ammonia and water, but Wieland showed conclusively that this was not the case and adduced evidence to show that the change was actually



The first stage, which represents the actual oxidation, is thus a simple dehydrogenation, and Wieland made the important observation that the oxidation of amino acids by charcoal proceeded equally well if dinitrobenzene were substituted for oxygen. (It will be remembered that this was one of the general hydrogen acceptors used for the investigation of the dehydrases.) It is clear, therefore, that the reaction is not brought about simply by the activation of the molecular oxygen and that the charcoal activates the amino acid very much like a dehydrase. The system will not reduce methylene blue, since the energy of the reaction is insufficient.

With regard to the dehydrases, while it is clear that the anaerobic dehydrases cannot react with oxygen in the absence of a special cyanide-sensitive catalyst (the nature of which will be made clear in the next section), it is equally clear that the aerobic dehydrases have no need of such a catalyst in order to react with oxygen. It was pointed out by Dixon and Thurlow (1925) and by Dixon (1927, 2) that the oxidations brought about by the xanthine oxidase form exceptions to Warburg's theory. They found not only that cyanide had no action, as mentioned previously,

but that the addition of iron or copper compounds produced no effect. Another exception is the tyramine oxidase.

With regard to the spectrum of the CO compound of the "respiratory enzyme," it seems doubtful if the experiments are to be interpreted in such a way, as Keilin points out. In photochemical reactions the presence of a coloured substance, the so-called sensitiser, is frequently necessary. The function of this substance is simply to absorb the light and make the energy available for the reaction. Now in such systems it is found that the effective light corresponds simply with the absorption spectrum of the sensitiser. It is possible that in the dissociation of the CO compound of the "respiratory enzyme" the ordinary haematin compounds of the cell act as sensitisers, and the curve observed is merely their spectrum and not that of the "respiratory enzyme." A number of reasons are given in the next section for believing that the "respiratory enzyme" is not a haematin derivative.

The oxidation of cysteine and glutathione is undoubtedly a metal catalysis, but the later work of Toda (1926) and Harrison (1927) has shown that the metal acts rather as an intermediate carrier than an oxygen activator, for the effects are still obtained when methylene blue is substituted for the oxygen. That is to say, the reduction of methylene blue by cysteine is likewise a metal catalysis, and is inhibited by cyanide and accelerated by adding iron or copper in traces. The metal simply seems to be reduced by the cysteine, and the reduced metal is then oxidised by the methylene blue or oxygen.

Finally, in considering the effect of cyanide on cell respiration it must not be forgotten that the cyanide poisons many systems besides the "respiratory enzyme," for instance peroxidases, catechol oxidase and its related enzymes, catalase, the glutathione system, etc. It does not follow that the whole of the cyanide inhibition of respiration is due to its action on the "respiratory enzyme."

These points of criticism leave the main point, the existence and importance of the "respiratory enzyme," unaffected. It is quite clear that a large part of the respiration is due to a catalyst which behaves very like an iron compound and is poisoned by HCN, CO, H<sub>2</sub>S, etc. It is, however, equally clear that other types of system, such as some of those previously considered, may contribute a by no means negligible part of the respiration.

The following section will deal with recent work on the nature and mode of action of the "respiratory enzyme" and its relation to the systems previously described.

#### V. THE CYTOCHROME SYSTEM.

The recent work of Keilin (1925; 1926; 1929) has done much to clear up the relations of the various types of oxidase system with one another.

Keilin (1925), by means of spectroscopic observations, showed the existence of a pigment, which he called "cytochrome," occurring in most living cells. This pigment can exist in an oxidised and a reduced form; the oxidised form shows only a rather indefinite spectrum, but the reduced form shows four strong absorption bands, which may be called *a*, *b*, *c*, *d*, beginning from the red end of the spectrum. The band *d* seems to be made up by the fusion of three bands lying close

together. Keilin showed that reduced cytochrome was not a single substance, but was composed of three very closely related components (*a*, *b* and *c*) resembling haemochromogens in spectrum and other properties but differing from them in a number of important respects. The spectrum of reduced cytochrome was therefore formed by the superposition of three similar two-banded spectra differing slightly in position, so that for instance the *a* component contributed the *a* band and one component of the *d* band, and so on.

By observing the change in the cytochrome spectrum, it is possible to follow its oxidation or reduction while it is actually present in the intact cell, and by such observations Keilin obtained many important results. If, for instance, a suspension of yeast cells in water is examined spectroscopically the four bands of reduced cytochrome are clearly seen. If now air is rapidly bubbled through the suspension the bands are no longer visible, that is to say, the cytochrome has become oxidised; but as soon as the bubbling is stopped, or if nitrogen is bubbled through instead of air, the bands immediately reappear. Similar results can also be obtained with animal tissues.

This means that oxidised cytochrome is rapidly reduced by systems in the cell, and reduced cytochrome is rapidly oxidised in the cell in presence of oxygen. In other words, the cytochrome must be acting as an intermediate carrier of the type mentioned previously, and on account of the rapidity of the process it may be expected to play a considerable part in the respiration of the cell.

Keilin then studied the action of inhibitors of respiration such as those used by Warburg. He found that the addition of the indifferent narcotics (*i.e.*, those organic substances which were supposed to act by a general blocking of the surface, and had no special action on iron) prevented the *reduction* of the cytochrome, and did not inhibit its oxidation. For instance, if ethyl urethane is added to a yeast suspension the cytochrome is immediately oxidised, and does not become re-reduced in absence of oxygen. This indicates that the indifferent narcotics act not on the oxygen activating system of the cell, but on its reducing systems.

On the other hand, if a small amount ( $M/10,000$ ) of cyanide is added to a yeast suspension, the cytochrome (*a* and *c*) immediately becomes completely reduced, and does not become oxidised, no matter how rapidly oxygen is bubbled through the suspension. Further, if air is bubbled through a yeast suspension so that the cytochrome is completely oxidised, and then a trace of cyanide is added, the cytochrome immediately becomes reduced, since the cyanide does not act on the reducing systems in the cell.

Keilin showed that reduced cytochrome *a* and *c* are not autoxidisable, even in presence of iron, wherein they differ fundamentally from ordinary haemochromogens; reduced cytochrome *b*, on the other hand, resembles these in being readily autoxidisable even in presence of cyanide. Therefore on aeration of a yeast suspension containing cyanide the component *b* still becomes oxidised although *a* and *c* do not. (For convenience in what follows the word cytochrome used alone will denote the components *a* and *c*, unless otherwise indicated.)

The facts just mentioned show clearly that the living cell contains systems capable



of oxidising reduced cytochrome by means of oxygen and of reducing oxidised cytochrome. It may be said that the indifferent narcotics inhibit the latter systems and do not affect the former, whereas cyanide inhibits the former only and has no action on the latter. It is therefore evident that the cyanide and the narcotics are acting on totally different systems—a conclusion of importance in relation to Warburg's work on inhibitors discussed in the previous section.

The cytochrome acts then as an intermediate carrier between the catalytic systems which reduce it and those which oxidise it, and its degree of oxidation or reduction at any instant will depend simply on the relative velocities with which these two reactions take place. Any factor which diminishes the activity of the reducing systems will send the cytochrome over more into the oxidised state, while factors which inhibit the oxidising systems will cause the cytochrome to become more reduced.

Keilin's later work (1929) was concerned with the properties and identification of the systems in the cell which reduce and oxidise cytochrome. He was successful in demonstrating the nature of both these types of system quite convincingly. It may be immediately stated here that the systems responsible for the reduction of cytochrome in the tissues proved to be, as would naturally be expected from what has been said above, the dehydrase systems. On the other hand, the catalyst responsible for the oxidation of reduced cytochrome by oxygen proved to be identical with the well-known indophenol oxidase which is so active and widely distributed in animal tissues, yeast, etc. For the full evidence of identification the original paper must be consulted, but a few of the more important points will be mentioned here.

In general it may be said that all factors which inhibit the dehydrases inhibit the reduction of cytochrome, and all factors which inhibit the indophenol oxidase inhibit the oxidation of cytochrome (*a* and *c*).

Considering the dehydrases first, these are inhibited by indifferent narcotics, which prevent the reduction of cytochrome; they are not inhibited by cyanide, which does not inhibit the cytochrome reduction: by washing a tissue with water the dehydrases become inoperative, owing to the removal of their substrates, and washing the tissue stops the reduction of cytochrome; and finally the activity of the dehydrases can be restored by adding various hydrogen donors, and these also restore to the washed tissue the power to reduce cytochrome.

In support of the last two points we may quote the behaviour of heart muscle which has been finely minced and well washed with water. This shows no spectrum, since the cytochrome is all in the oxidised form because the hydrogen donors have been removed and there is therefore nothing present to keep it reduced. If, however, a trace of reducing agent such as sodium hydrosulphite is added, the bands of reduced cytochrome immediately appear. On shaking with air the bands disappear, owing to the oxidation of the cytochrome by the indophenol oxidase present. If, however, a trace of cyanide is added in addition to the hydrosulphite, the bands remain, as the indophenol oxidase is poisoned by the cyanide.

If now to a sample of the washed tissue, in which there is no tendency for the cytochrome to become reduced, a small amount of succinate is added, the cytochrome

becomes reduced in about 15 seconds, owing to the presence of the succindehydrase. This reduction is in opposition to the oxidising action of the indophenol oxidase, and if this is abolished by the addition of a little cyanide before the succinate the reduction of the cytochrome by the succinate becomes *instantaneous*. It is clear, therefore, that in the absence of cyanide a continuous reduction of cytochrome by the dehydrase and oxidation by the indophenol oxidase must be going on, resulting in a continuous uptake of oxygen and oxidation of the succinate. Now we already know that this is actually observed, from the experiments of Fleisch quoted previously in the section on the dehydrases, and we know also that this uptake is stopped by a small amount of cyanide. Keilin's work then provides the explanation for the effect of cyanide on the succinoxidase system, which was previously somewhat puzzling. We conclude then that the succindehydrase, like the lactic and citric dehydrases, is unable to react directly with molecular oxygen; that the action of the cyanide on the system is due to its action on the indophenol oxidase, which is concerned with the actual utilisation of molecular oxygen; and that the cytochrome acts as an intermediate carrier between the dehydrase and this oxidase. Keilin showed that preparations of the succinoxidase made from muscle also contained cytochrome and indophenol oxidase.

Results similar to those given by washed muscle were also obtained with yeast. Here lactates were especially active as hydrogen donators, in agreement with results obtained by the use of methylene blue.

Turning now to the action of various factors on the indophenol oxidase, we may mention that it is inhibited by KCN in traces,  $H_2S$ , CO, ethyl cyanide, drying the cells, etc., and all these show a corresponding effect on the rate of oxidation of cytochrome. The narcotics, etc., which do not affect the indophenol oxidase do not affect the oxidation of cytochrome. Keilin has recently directly shown the oxidation of a solution of reduced cytochrome *c*, extracted from yeast, by a preparation of indophenol oxidase.

Indications have not been wanting in what has been said above that there is a close relationship between the indophenol oxidase and the "respiratory enzyme" of Warburg. For instance, they are both enzymes concerned with making molecular oxygen available for cell-oxidations, and are both extremely sensitive to cyanide and  $H_2S$  and also inhibited by carbon monoxide. Keilin was led by this similarity to make a special study of the properties of the indophenol oxidase of yeast and mammalian heart muscle in comparison with those of the "respiratory enzyme," and as a result he showed convincingly that the "*respiratory enzyme*" is identical with indophenol oxidase. The full evidence for this important identification (which Warburg (1929) appears to accept<sup>1</sup>) is given in Keilin's paper (1929), but the following summary of some of the main characteristics of the indophenol oxidase as found by Keilin will show how closely they resemble those of the "respiratory enzyme."

*The indophenol oxidase.* In addition to cytochrome, this enzyme is able to oxidise

<sup>1</sup> Warburg claims that the indophenol oxidase should be called the "respiratory enzyme." The name indophenol oxidase is admittedly unsatisfactory, but it was in common use long before the conception of the "respiratory enzyme" had been introduced. Also the "respiratory enzyme" is not the only enzyme responsible for respiration. Perhaps the name "cytochrome oxidase" would be preferable.

the indophenol ("nadi") reagent ( $\alpha$ -naphthol + dimethylparaphenylenediamine), giving indophenol blue (Röhm and Spitzer, 1895); and also paraphenylenediamine itself (Batelli and Stern, 1912, 1) to the diimine, which then becomes converted into dark coloured products. These reactions therefore furnish two alternative methods of investigating the oxidase. In the first method, which is convenient for qualitative work, the rate of appearance of the blue colour in the presence of oxygen is observed. In the second method, which gives accurate quantitative results, the rate of oxygen uptake is measured after the addition of paraphenylenediamine.

Keilin has investigated the indophenol oxidase of yeast and heart muscle by both these methods. He found that cyanide, in concentrations of the same order as those required to inhibit respiration and the oxidation of cytochrome, inhibited both the production of indophenol blue and the oxidation of *p*-phenylenediamine.  $H_2S$  had the same powerful inhibitory effect on all. Pyrophosphate, which also inhibits many iron-catalysed reactions, had, on the other hand, no effect, as also narcotics such as ethyl urethane.

The experiments with carbon monoxide are particularly interesting. Keilin showed that CO inhibited both the indophenol and *p*-phenylenediamine oxidations partially, that in both cases light prevented the inhibition, and that in both there was a definite "competition" between the oxygen and the CO. All these are characteristic of the "respiratory enzyme." By means of the *p*-phenylenediamine technique Keilin made a large number of measurements on the relative affinities of the indophenol oxidase for oxygen and CO. As mentioned in the previous section, Warburg had worked out the relative affinities for the "respiratory enzyme" from direct measurements on respiration, and expressed the results in the form of a relative affinity constant which had a value of the order of 10. Keilin's individual values varied somewhat, like Warburg's, but the mean value of the constant for the indophenol oxidase he found also to be about 10. (It tended to be slightly higher with yeast, and slightly lower with heart muscle.) This means, of course, that in any mixture of CO and oxygen the percentage inhibition of the "respiratory enzyme" and the indophenol oxidase are always the same, and constitutes very strong evidence of identity. This is all the stronger as the other known substances which combine with CO have constants of a very different order of magnitude.

It may be mentioned that none of the three forms of cytochrome combines with CO, therein showing an important difference from the ordinary haemochromogens.

Keilin compared these properties of the indophenol oxidase with those of the somewhat analogous catechol oxidase of the potato, which was discussed in a previous section. He found that the oxidation of catechol by this enzyme was likewise inhibited by cyanide,  $H_2S$  and CO. In this case also there was a definite competition between the oxygen and the CO. The value of the constant, however, was very different from that for the indophenol oxidase, being slightly over 1. The catechol oxidase is therefore more strongly inhibited by CO. An interesting fact is that light had absolutely no effect on the combination of this enzyme with CO. It was suggested above that such effects might depend on the presence of haematin compounds

acting as photochemical sensitisers, but whether this is capable of explaining the difference is not yet certain.

Taking then the identification of the "respiratory enzyme" with indophenol oxidase as being satisfactorily established, it may be said that Keilin's work has made clear the function of the "respiratory enzyme." The anaerobic dehydrase systems are unable to react directly with molecular oxygen. They react readily however with the intermediate carrier cytochrome, and the "respiratory enzyme" then brings about the reaction between cytochrome and oxygen. On the classification given in the introduction this forms a system of type 4 with an intermediate carrier.

The ability to act as a carrier in this system seems to be a special property of cytochrome, and not a general property of haematin compounds. Keilin found that other haematin compounds were not reduced by the succindehydrase system in several hours, while cytochrome was reduced by the same system in a few seconds.

Although it seems fairly certain that in the case of yeast the cytochrome-indophenol oxidase system is responsible for practically the whole respiration, the work of Dixon and Elliott (1929), referred to in the previous section, shows that this is not the case with animal tissues, since about one-third of the respiration is not inhibited by cyanide. It is not yet clear how much of the cyanide-sensitive part of the respiration of animal tissues is to be ascribed to the action of this system. This point will be discussed later.

Warburg believed, as stated above, that the "respiratory enzyme" was a haematin compound. Keilin, however, gives a number of reasons for believing that this is not the case<sup>1</sup>.

(1) It is inhibited by very small traces of KCN. But KCN in traces has no effect on the oxidation of reduced haematin or haemochromogens, and in higher concentrations it combines with them making them more autoxidisable.

(2) It is inhibited by  $\text{H}_2\text{S}$ . But  $\text{H}_2\text{S}$  does not combine with these compounds, and does not affect their oxidation.

(3) CO and  $\text{O}_2$  compete for the "respiratory enzyme." But no case of such competition for a catalytically active haematin compound is known. The oxidised ("ferric") haematin compounds do not combine with CO. The reduced ("ferrous") compounds (with the exception of cytochrome) have a very great affinity for CO; and moreover the CO then competes, not with  $\text{O}_2$ , but with the nitrogen compound forming the haemochromogen. The only case where there is a definite equilibrium between CO and  $\text{O}_2$  is that of haemoglobin, which does not undergo oxidation but oxygenation, and the iron of which is catalytically inactive.

(4) All haematin compounds show a peroxidase activity. The catechol oxidase, upon which KCN,  $\text{H}_2\text{S}$ , and CO have a very similar inhibiting action, shows no peroxidase activity, even when highly concentrated.

(5) The haematin or haemochromogen compounds cannot oxidise reduced cytochrome.

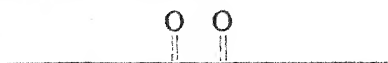
In short, the properties of the "respiratory enzyme" are not those of a haematin

<sup>1</sup> Points (1) and (3) have been briefly discussed by Warburg (1929).

compound but those of the indophenol oxidase. This of course does not imply that it does not contain iron.

We now come to the question of the actual mechanism by which the indophenol oxidase acts. Does it activate the oxygen or the cytochrome, or both? This question cannot be definitely answered as yet. The enzyme has not yet been obtained free from other enzymes, and its specificity has not yet been properly investigated. The few facts available do not permit of any final conclusions being drawn.

In the first place, the existence of a definite competition between CO and oxygen is in favour of an oxygen activation by the enzyme,<sup>1</sup> for it clearly means that the oxygen molecules are actually combining with the enzyme to form an active compound. Possibly the state of an oxygen molecule combined at the enzyme surface might be represented somewhat as follows



where the horizontal line represents the enzyme surface. Oxygen is known to be adsorbed in this way on certain surfaces. The activation is due to the molecule becoming virtually resolved into atoms, and this makes it possible for the oxygen to become reduced directly to water without the intermediate formation of hydrogen peroxide.

On the other hand, oxygen activation alone is clearly not sufficient to explain the action of the system, for if the oxidation of *p*-phenylenediamine were solely due to the production of active oxygen by the enzyme the active oxygen should also oxidise the analogous easily oxidised compounds catechol and hydroquinone. The indophenol oxidase is however quite unable to bring about the oxidation of either of these compounds, thus contrasting with the similar enzyme catechol oxidase, which will oxidise catechol but not *p*-phenylenediamine. The non-oxidation of substances like catechol by the indophenol oxidase would seem almost to indicate that active oxygen is not present.

At any rate the specificity of the oxidase towards the reducer, and particularly the differences just mentioned between the actions of the catechol and indophenol oxidases, would certainly seem to indicate that the enzyme acts by activating the reducer. But in that case a difficulty is introduced by the fact that it will also oxidise the totally different substance cytochrome, so that we have to explain why the oxidase which oxidises *p*-phenylenediamine will not oxidise the very similar substances catechol and quinone, while it will oxidise the totally different substance cytochrome. Part of this difficulty might be met by supposing that the indophenol oxidase does not oxidise cytochrome directly. For instance, the catechol oxidase will not oxidise cytochrome by itself, but will do so if catechol is added. Presumably the cytochrome is oxidised by the quinone formed. Similarly it might be supposed that the indophenol oxidase preparations contained some substance related to

<sup>1</sup> It cannot however be regarded as proof of oxygen activation. To take an analogy, in the oxidation of aldehyde by methylene blue in presence of the Schardinger enzyme, uric acid and methylene blue appear to compete for the enzyme; but the conclusion is not to be drawn that the enzyme activates methylene blue (which is already active). Nevertheless the analogy is perhaps somewhat superficial, and the probabilities seem to be as stated in the text.

*p*-phenylenediamine, and that the oxidation product of this was responsible for the oxidation of the cytochrome. But while this would meet the difficulty with regard to the specificity of the enzyme, we should then have to account for the specificity in the action of the oxidation product of the substrate.

It is clear that no definite answer can at present be given to the important but puzzling question as to the mechanism of the indophenol oxidase action. The matter must be left entirely open; but it is to be hoped that further work will soon throw some light upon it. In the meantime the commonly made assumption that the process is simply one of oxygen activation seems to be supported by insufficient evidence.

Before closing this section it should be mentioned that cytochrome, in common with other haematin compounds, has a powerful peroxidase activity. This may be an additional function of cytochrome in the cell.

## VI. GLUTATHIONE.

The glutathione system has already been reviewed in this journal (Tunncliffe, 1926), and therefore it will not be dealt with at length here. A brief summary of the main facts will however be given for the sake of completeness and to include recent work. Glutathione was isolated by Hopkins (1921) who showed that it was very widely distributed in living cells. He then believed it to be a dipeptide containing cysteine and glutaminic acid, but he has recently (Hopkins, 1929) obtained it in the pure crystalline state, and shown it to be a tripeptide consisting of cysteine ( $\text{CH}_2\text{SH} \cdot \text{CHNH}_2 \cdot \text{COOH}$ ), glutaminic acid and glycine. As it is the sulphur group alone which is of importance in connection with tissue oxidations, its formula may conveniently be abbreviated to GSH.

It readily undergoes oxidation to a disulphide form thus



GSH and GSSG are referred to as reduced and oxidised glutathione respectively.

Its importance in the cell arises mainly from the fact that the cell contains both systems which vigorously reduce oxidised glutathione and systems which rapidly oxidise reduced glutathione by means of molecular oxygen. That is to say, glutathione forms a carrier acting in somewhat the same way as cytochrome. We may consider first the systems which reduce glutathione, and then the systems which oxidise it. (It must be remembered that most of the work has been done with the non-crystalline, impure glutathione, but it is unlikely that any serious corrections will have to be made as a result of repetition with the pure material.)

*Reduction.* Although reduced glutathione is rapidly oxidised in the tissues, glutathione exists in them mainly in the reduced condition. This is because the oxidised form is undergoing reduction at the same time; and indeed if GSSG is added to tissues it becomes rapidly reduced to GSH.

One naturally thinks of the dehydrase systems as being responsible for this reduction. This, however, is not correct: none of the known dehydrase systems is capable of reducing glutathione in the slightest degree (Hopkins and Dixon, 1922).

It seems indeed that enzyme systems are not involved in the reduction, for the



reaction is unaffected by heating the tissue to boiling point for some time (Hopkins and Dixon, 1922; Bernheim and Dixon, 1928). The nature of the systems or substances which reduce glutathione in the tissues is quite unknown. Hopkins and Dixon showed that part of the reduction was due to "fixed —SH groups," *i.e.*, —SH groups which cannot be washed away from the insoluble protein residue of the tissue, and appear to form part of the structure of the protein molecules. These groups readily reduce added *GSSG*. This fact however merely carries the problem a stage further back, as the question then arises as to what maintains the fixed —SH groups in the reduced state. The fixed groups, if indeed they are essential to the system, act as carriers between the unknown substances oxidised and glutathione itself. It seems quite possible however that the glutathione may react directly with the oxidisable substance without the intervention of the fixed sulphur groups, and that the presence of the latter is merely fortuitous. Bernheim and Dixon found that samples of washed liver which contained no —SH groups, fixed or free, readily reduced *GSSG*, and that the reaction was unaffected by previously boiling the tissue. Until we know the nature of the substances oxidised by the glutathione system in this way the biological significance of the system will not become clear.

*Oxidation.* Ordinary reduced glutathione in neutral solution is autoxidisable, and it was therefore at first thought that *GSH* took up oxygen in the cell by a simple autoxidation. It was shown however by Harrison (1924), following the work of Warburg and Sakuma (1923) on cysteine, that iron-free glutathione is not autoxidisable, and that the oxidation was in fact due to an iron (or copper) catalysis. This however did not seriously affect the position, as the cell contains a considerable amount of iron in various forms, and also a certain amount of copper. In addition to iron and copper salts, iron in organic combination in the form of haematin is also active, as shown by Harrison. This observation is important in view of the presence of haematin compounds within the cell. (It appears from work by Dixon and Meldrum at present in progress that the presence of iron or copper alone is not sufficient to make pure glutathione autoxidisable, and the presence of a third substance is also necessary. The nature of this factor is not yet quite certain.)

It was shown by Thurlow (1925) and by Harrison and Thurlow (1926) that peroxide was formed during the aerobic oxidation of —SH compounds in presence of iron. The amounts were too small to be detected chemically, but they could produce coupled oxidations of nitrite in the presence of peroxidase, or of lactic acid, etc., in presence of ferrous salts. *GSH* is therefore an "oxygenase." It seems however somewhat doubtful whether this is an important function of glutathione.

The exact significance of Szent-Györgyi's (1928) hexuronic acid system, previously mentioned, seems somewhat obscure. If it acts simply as a carrier between glutathione and peroxidase it would merely provide a method of oxidation of glutathione alternative to that described above. But since both hexuronic acid and the polyphenol necessary for its action occur only in very small amounts, if at all, in most animal tissues, it seems very questionable whether this oxidation is significant in comparison with that due to iron-containing systems. Hexuronic acid probably has other functions.

*Other functions of glutathione.* Glutathione is able to bring about the oxidation of biological substances in other ways than by acting as a carrier in the manner just described, which seems to be its chief function. Its action as a carrier depends of course on the alternate oxidation and reduction of its sulphur groups. GSH can however act as a catalyst of oxidation by mechanisms which do not involve any oxidation of its —SH group, and indeed under conditions in which this group is not autoxidisable. (For a full account of such reactions see Hopkins (1925). See also Allott (1926).)

GSH in moderately acid solutions is not autoxidisable, even after the addition of a trace of iron. Yet on the addition of a little GSH to an acid emulsion of unsaturated fats or fatty acids a steady and continuous oxygen uptake commences, and continues until amounts of oxygen have been absorbed equivalent to many times that necessary to oxidise the GSH present, which is still found in the reduced state. Not only is the —SH group not oxidised under these conditions but unsaturated fats or fatty acids are incapable of reducing GSSG. Moreover the glutathione must be added in the reduced form, the disulphide form being inactive. In neutral solution somewhat similar effects are observed but here the —SH group undergoes oxidation at the same time, and when this is all oxidised the reaction ceases. If, however, some extra system is added which can maintain the glutathione in the reduced state, *e.g.*, a protein containing fixed —SH groups, it is possible to obtain extended oxygen uptakes with oxidation of the fat in neutral solution. The phenomena are complex, but it is clear that GSH is acting as a catalyst directly between the unsaturated fat and molecular oxygen. The mechanism by which it acts is however quite unknown, and it is also uncertain how far iron complexes may be playing a part in the process.

A somewhat similar case is the oxidation of proteins. If to a protein containing fixed —SH groups in its structure a little GSSG is added at neutral reaction an uptake of oxygen commences. Neither the protein nor GSSG takes up oxygen separately. The reaction is primarily due to a reduction of the glutathione by the fixed —SH groups, and the uptake is then due to the autoxidation of the resulting GSH. The total amount of oxygen taken up corresponds however to more than 10 times the equivalent of the fixed —SH groups originally present, so that it is clear that by far the greater part of the oxygen has been used for the oxidation of the protein itself in addition to its fixed —SH groups. The fixed —SS— groups formed can again be reduced, and again, in presence of GSSG, an oxygen uptake of 10 times the expected amount is obtained. The mechanism of this protein oxidation is also unknown, but it seems to depend in some way upon the oxidation of the —SH groups. It does not occur in acid solutions. The significance of these reactions awaits further investigations.

## VII. CONCLUSION AND SUMMARY.

We have now considered the various known types of oxidation-catalysts present in animal tissues—the dehydrases, the peroxidases, indophenol oxidase, cytochrome, glutathione; and we have also considered their relations with one another. We arrive at the following picture of the events in the tissues.

The various organic materials to be oxidised are activated by a series of enzymes—the dehydrases. These are of two types, those of one class—the aerobic dehydrases—are able to oxidise their substrates by direct reaction with molecular oxygen; those of the other class—the anaerobic dehydrases—are for some reason not able to do so.

The aerobic dehydrases, in oxidising their substrates, reduce the molecular oxygen first to hydrogen peroxide and then to water. The hydrogen peroxide may be activated by peroxidases (or haematin compounds) and so oxidise directly a number of organic substances.

The anaerobic dehydrases oxidise their substrates by reducing cytochrome. The cytochrome reduced in this process is oxidised by indophenol oxidase with uptake of oxygen.

Further investigation is necessary before we can say definitely how the glutathione system is connected with the other systems.

This picture is of course liable to be modified by subsequent investigation, but it appears to be strongly supported by the present state of the evidence. It seems likely that other oxidases will be discovered.

According to this scheme, cell oxidations proceed along two main lines initiated by the two types of dehydrase respectively. Corresponding to these two lines the absorption of oxygen (respiration) is due to two different processes. In one the oxygen oxidises cytochrome under the influence of indophenol oxidase; in the other the oxygen is converted into hydrogen peroxide in oxidising the substrates of the aerobic dehydrases, tyramine oxidase, etc.

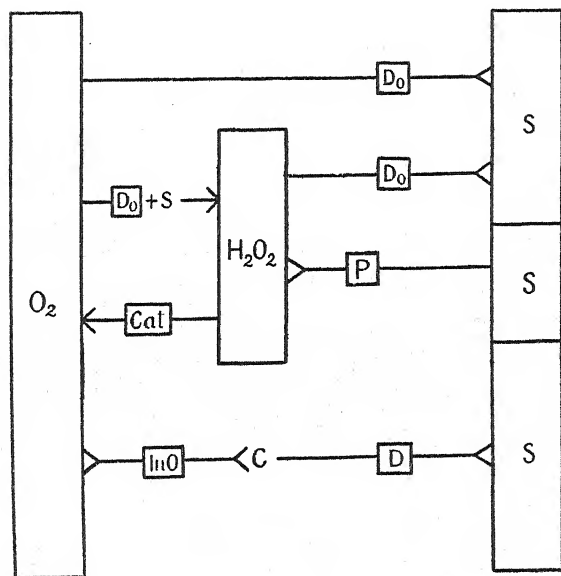
Making the fairly probable, but perhaps not absolutely justifiable, assumption that the respiration of tissues consists entirely of these two processes, we can obtain some information as to their relative importance in respiration from the effect produced by cyanide. Cyanide does not inhibit the aerobic dehydrases and those systems which react directly with oxygen, but it inhibits completely the indophenol oxidase system. Dixon and Elliott find that cyanide inhibits the respiration of animal tissues from 50 per cent. to 80 per cent. in most cases. It seems therefore that the cytochrome system cannot contribute more than about two-thirds of the total respiration, the remaining third being presumably due to the aerobic dehydrases and similar systems. (The xanthine oxidase by itself cannot account for this fraction, since it is absent from muscle where there is a considerable cyanide-stable fraction.)

The absorption of oxygen by the glutathione system is prevented by cyanide, and it must therefore be included, with that due to the cytochrome system, in the cyanide-sensitive fraction of respiration.

Warburg, confirmed by Dixon and Elliott, found that practically the whole

respiration of yeast was cyanide-sensitive; and indeed his experiments on the effect of CO show that almost all the oxygen uptake was due to the action of the indophenol oxidase. This does not indicate that aerobic dehydrases must be absent from yeast. Dixon and Elliott found indeed that the cyanide-stable respiration of yeast was, weight for weight, definitely greater than that of animal tissues. The respiration due to the indophenol oxidase is however more than fifty times as great in yeast as in animal tissues, so that the cyanide-stable part becomes relatively insignificant.

With regard to the possibility of the autoxidisable haematin compounds acting as carriers between oxygen and the anaerobic dehydrases, so as to enable the latter to act even when the indophenol oxidase has been poisoned by cyanide, two facts make this rather unlikely. In the first place, the oxidation of succinate in tissues is completely stopped by cyanide. If the haemochromogens (or cytochrome *b*) were



able to act as carriers in this system this should not be the case, since the cyanide does not interfere with their autoxidation in the tissues. Secondly, Keilin has shown that the ordinary haematin compounds are only reduced with extreme slowness, if at all, by the anaerobic dehydrases. It is of course possible that they may act as carriers with other systems, but cytochrome appears to be particularly specialised for this purpose.

It may be convenient to summarise in the form of a diagram the relationships of the various systems and the various ways in which the organic substrates of oxidation are caused to react in the tissues. In the above diagram the *main* groups of oxidase systems occurring in the tissues are indicated in their relation to one another.

In this diagram the following symbols are used. *S* represents the various

substrates of oxidation, *i.e.*, the organic substances undergoing oxidation. *D* represents the (anaerobic) dehydrases, and *D*<sub>0</sub> the dehydrases which can utilise oxygen (the aerobic dehydrases). *P* represents peroxidases (and haematin compounds acting as peroxidases). *Cat* denotes catalase, *InO* indophenol oxidase (= the "respiratory enzyme"), and *C* cytochrome. For the sake of clearness enzymes are enclosed in small squares. The symbol  $A \xrightarrow{[B]} C$  should be read "*A*, being activated by the enzyme *B*, reacts with *C*"; and  $A \xrightarrow{[B]} C$  signifies "*A*, under the influence of *B*, is converted into *C*."

The top line represents reactions such as the oxidation of hypoxanthine, etc., by the xanthine oxidase. The second line shows on the left the formation of hydrogen peroxide during these reactions, and on the right its subsequent reduction to water by the same system. Below this is represented the various coupled oxidations in presence of peroxidase, and on the left the protective action of catalase. These systems are connected with the first main line of events, where oxygen acts as a direct hydrogen acceptor, and perhaps account for about one-third of the total oxygen uptake.

The second main line of events, accounting probably for a considerable part of the remainder, is represented by the indophenol oxidase oxidising cytochrome (the mechanism is largely speculative—the activation of both reactants is represented), which in turn is reduced by the anaerobic dehydrases plus their substrates. This chain of events is prevented by cyanide, etc.

An attempt has been made in this article to give a fairly general survey of the present position of the subject, and in particular to show the co-ordinating influence of work done in the last few years. It is of course impossible in such an article to mention all the modern work on the subject, but an endeavour has been made to give some account of all the more important recent developments. In a subject advancing as rapidly as that under consideration it cannot be expected that the current views will remain unmodified for long. Nevertheless it is hoped that those put forward here are sufficiently well grounded not to be seriously shaken by future developments.

One fact will be evident, namely, that very little progress has been made in linking up the knowledge gained from the study of these oxidation mechanisms with that gained from work on intermediary metabolism. The time seems ripe for such a line of progress. We know of the existence in the tissues of many oxidation systems, and we have some idea of their mode of action; but frequently, as in the cases of peroxidase and glutathione, we are ignorant of the nature of the substances they oxidise. We know from work on intermediary metabolism of a considerable number of oxidative changes which occur in the tissues, but we are ignorant of the nature of the systems which bring about the particular changes. It may be expected that investigations on such lines will yield many interesting and important results.

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